Macro- and microecological succession in wetlands following major disturbance

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Abstract: Destruction of wetlands continues at an alarming rate, with loss of almost half of the wetlands in the continental United States. The severity and persistence of environmental effects due to wetland destruction are largely unknown. This research project was designed to examine the influence of wetland disturbance on three of its major features: vegetation, soil chemistry, and soil microbial diversity. Three wetland sites were selected: i) an area of a wetland that has been disturbed by the construction of an underground oil pipeline (referred to as Disturbed); ii) the other half of the same wetland, which has not been directly disturbed (Undisturbed); and iii) a wetland in close proximity to act as a positive control (Larue). Vegetation and soil sampling was executed in three seasons (spring, summer, and fall) over a period of three years (2004-2006). The majority of new species acquired in the Disturbed wetland were non-native and/or invasive and noxious. Significant differences in soil chemistry of the Disturbed wetland were clearly evident: soil pH significantly increased ($P < 0.0001$) while levels of P, B, Zn, Fe (lbs/acre), NH_4-N (ppm), % organic matter, % moisture, and cation exchange capacity were all significantly decreased ($P < 0.0001$). The Disturbed wetland also had lower water permeability. Soil microbial diversity was examined by using a combination of Polymerase Chain Reaction and Denaturing Gradient Gel Electrophoresis (PCR-DGGE). All three sites showed variations in DGGE profile with respect to season and year, however, the results clearly indicated that the soil of the Disturbed wetland had lower diversity compared to the other two sites; and these differences were maintained throughout the 3-year sampling period. Collectively, these findings show that the disturbance of the wetland, and the ensuing soil treatments (fertilizer and sowing) altered wetland chemistry, vegetation and microbial diversity. Furthermore, these practices were not effective in returning the wetland to its previous “undisturbed” state, at least within the three years after the disturbance. Other strategies need to be considered if we are to lessen the

medium- to long-term impacts of wetland disturbance and destruction, and to create more effective wetland restoration strategies.

INTRODUCTION

Wetlands provide numerous beneficial ecological functions including: water purification; retention and/or removal of particulates, elements, and compounds; flood protection; recharging the groundwater supply; maintaining stream flow; nutrient cycling; and providing fish and wildlife habitat (Smith et al. 1995). However, despite these benefits, the loss of wetlands continues to be enormous. In the lower forty-eight states, almost half of all the wetlands have been destroyed (Dahl and Allord 2002).

Wetland vegetation (hydrophilic vegetation) is an often-used indicator to determine if an area can be classified as a wetland, and is a prime candidate for study to determine changes to a wetland that is caused by disturbance. Because of the hydrologic and geologic conditions of a wetland, plants found in wetlands experience water at, or near the surface of, the soil for at least some period during the growing season. Therefore, plants found in wetlands are normally distinct from upland plants, with wetland plants having some adaptations to living in saturated soil conditions. When water fills the pores in the soil, anaerobic conditions can result. These conditions prevent plants from carrying out respiration through their roots, affects the availability of nutrients, and the amount of toxic materials present (Miller and Zedler 2003). Thus, wetland plants must be adapted to survive in these conditions. Wetlands also have high soil organic matter, which supply the vegetation and microbes with nutrients, especially nitrogen (which is usually the limiting nutrient for plant growth), phosphorus (vital for plant growth), and sulfur (common in wetlands). Other nutrients include iron, which is oxidized to its ferric form in aerobic soils and reduced to its ferrous form in anaerobic conditions. Some iron-oxidizing bacteria can convert ferrous iron to ferric iron, and high concentrations of ferrous iron can be toxic to plants, forming iron oxide around plant roots and preventing nutrient uptake (Singer and

Munns 2006). These iron oxide deposits have also been found to contain abundant microbes (Weiss et al. 2002). Studies have shown that seasonal changes in environmental conditions can result in different concentrations of trace elements. The dissolved concentrations of Fe, Mn, Al, Cu, Zn, La, U, Th, Cd, and As in wetlands resulted in significant changes over seasons for all of these elements except Zn and Cu, with low concentrations until a sharp increase in mid-February. This increase is believed to occur because of a decline in redox potential along with an increase in carbon via soil organic matter (Olivie-Lauquet et al. 2001). As such, the microbiology of wetland sites, and their interactions with soil chemistry, can have a large influence of the types of plants that propagate and develop in a wetland community.

Microbes in wetlands have most often been studied in context to nutrient cycling but few studies have been conducted on microbial changes in the soil after a disturbance. Wetlands contain large organic matter concentrations (organic soils have more than 20 to 30% organic materials and wetlands soils may contain up to 90% organic materials (Singer and Munns 2006)) and it is probable that the microbes have adapted unique characteristics to survive in low dissolved oxygen environments. Some microbes prefer low dissolved oxygen concentrations (microaerophiles) while others are anaerobic and would thrive in a saturated soil environment. Recent studies have shown that as decomposition of plant residues proceeds, there is a concurrent increase in microbial diversity (Dilly et al. 2004). Enrichment of nitrogen in wetlands, for example, typically results in a large and immediate increase in microbial abundance. High concentrations of heavy metals, such as copper, result in decreased microbial richness, as does acidity in wetlands that have not been adapted to acidic conditions for a significant period of time. However, long-term accumulation in soils of some heavy metals, such as zinc, can result in a tolerance of the microbes towards zinc and not necessarily lead to a

decrease in the survival of microbes (Davis et al. 2004). Other factors that may affect the microbial communities of wetlands, but on which few studies have been conducted, are temperature, salinization, sedimentation, turbidity, shade, removal of vegetation, dehydration, and inundation (US EPA 2006). In other words, wetland disturbances that create major perturbations in nutrient availability can affect soil microbial diversity and as a result, alter the macrobiological characteristics, form, and function of the wetland.

As outlined above, disturbance causes many significant changes to the composition of a wetland. Vegetational changes are perhaps the most obvious, but the entire ecology of the wetland, such as wildlife habitats and food supply, erosion, and temperature can also be changed. Changes in the soil can also result in major shifts of minerals, elements, and microbes, which will in turn affect other aspects of the wetland, such as vegetation, acidity, and heavy metal concentrations. It is important to study and become more aware of the consequences, both primary and secondary, that may occur after the disturbance of a wetland, if better and more effective strategies for wetland restoration are to be developed.

In the fall of 2003, the construction of a highly controversial 149-mile underground oil pipeline was completed from Kenova, West Virginia to Columbus, Ohio, crossing 363 streams and 55 designated wetlands (US Army Corps of Engineers 2001). This study encompasses one of the wetlands through which the pipeline was constructed with comparative analyses made to another wetland in close proximity that was undisturbed by the pipeline construction. To my knowledge, this is one of only a few studies that have investigated the impacts of wetland disturbance on the macroecology and microbial ecology, and how perturbation of the macro- and microbiology affects wetland characteristics and sustainability.

I hypothesized that by following the changes in wetland vegetation, soil chemistry, and soil microbial community structure after major disturbance, then its impact on macro- and microecological succession will be determined.

To address my hypothesis, my objectives are to examine and obtain biological and chemical data for three wetland areas: i) an area of a wetland that has been disturbed by the construction of an underground oil pipeline (referred to as Disturbed); ii) the other half of the same wetland, which has not been directly disturbed (Undisturbed); and iii) a wetland in close proximity to act as a positive control (Larue). For each area, I compared vegetation, soil chemistry, and microbial community structure across sites and through time.

Relationships among changes or otherwise in the soil microbes, the soil chemistry, and the vegetation are investigated, from which better and more effective strategies for wetland restoration will hopefully be developed.

METHODS

Overview

Three wetland areas were selected for monitoring over three years (2004-2006), one is half of a wetland that has been disturbed by the construction of an underground pipeline (in which the topsoil was not segregated and not placed back on the top after construction was complete [Disturbed]), the second is the other half of the wetland that was not directly disturbed (Undisturbed; note also that both of these areas comprise the Sherman wetland), and the third is a wetland in close proximity, approximately 0.4 km northwest (Larue wetland). This third wetland serves as a positive control to ensure that the results are not due to some outside environmental factors, such as a severe drought, extreme temperatures, or above average precipitation.

Vegetation Analyses

The construction of the pipeline was completed in the fall of 2003 and the first vegetation surveys for both Disturbed and Undisturbed was taken immediately afterward to use as a baseline. Using the sampling technique for vegetation, developed and used by the Environmental Protection Agency, each wetland was mapped. The entire grid was five squares by two squares, with the centerline running north-south. Each square was 10 m by 10 m. The furthest south-east square was numbered one and consecutive numbers were assigned going counter-clockwise. In the Sherman wetland, squares one, two, three, eight, nine, and ten fell in the undisturbed portion of the wetland, and squares four, five, six, and seven fell in the disturbed portion. In the Larue wetland, all ten squares were in the undisturbed control area (Appendix A, Figures 1 and 2). A survey of all the non-woody vegetation, (recording presence and location, not amount or size) identified to genus, and if possible, species, in all the squares was taken in the fall of 2003 for Disturbed and Undisturbed Wetlands. In following years, the vegetational

surveys were continued three times a year, in spring, summer, and fall. The three years of sampling results, plus the baseline, were compared and the changes in vegetation resulting from the disturbance, either directly or indirectly, were determined.

Soil Chemistry Analyses

For the soil analysis portion of the experiment, three soil samples were taken from each of the three different areas. The three sample sites were chosen by picking out a relative wet area (#1), a semi-wet area (#2), and a dry area (#3), and then randomly choosing the actual sampling site. The sampling sites were marked to ensure that the same area was sampled in consecutively times. There was a total of nine different soil sampling sites, six in the Sherman wetland (three in Disturbed, three in Undisturbed), and three in Larue (Appendix A, Figures 1 and 2). For each sampling, an adequate amount of soil was removed using a soil probe (an eight inch core sample was extracted multiple times), mixed together, and then a small, air-tight tube was filled for the microbial analysis. These tubes were then frozen at –80^{o}C while awaiting further analysis. The rest of the soil was placed in soil testing bags provided by the CLC Lab (Westerville, OH) for the chemical analysis. These were sent to the CLC Lab and tested for pH, P, K, Ca, Mg, Z, Mn, Cu, Fe, S, B, and NO_3 (lbs/acre), % organic matter, cation exchange capacity, K, Ca, Mg, (% base saturation), and NH_4-N in ppm. Sampling occurred for three years, with three samples from three areas in three seasons for three years equaling a total of 81 soil samples for both the chemical analysis and the microbial analysis (Appendix A, Table 1).

Percent Moisture Analyses

Each sample (1-81) was analyzed for percent moisture by weighing out 5 g of soil into aluminum weighing dishes. Each sample was oven dried (40.5 ^{o}C) for 24 hrs, placed in a

desiccator until cool, then reweighed. The difference between the wet and dry weight was divided by the wet weight to obtain percent moisture.

Soil Permeability Analyses

In the fall of the third year of the study (2006), soil permeability was tested in all three wetland areas: Disturbed, Undisturbed, and Larue. A soil core sample of at least 8 cm in height and 7 cm in diameter was collected at each site and permeability tests were completed by Hull & Associates, Inc., using either a constant head or falling head test.

Soil Microbial Analyses

DNA Extraction.--

Soil DNA extraction was achieved using the Repeated Bead Beating Plus Column (RBB+C) Method (Yu and Morrison 2004*b*). In brief, for cell lysis, 0.5 g of soil sample was added to lysis buffer and zirconia beads. This was homogenized using a Mini-Beadbeater, incubated at 70^{o}C, and centrifuged. The supernatant was transferred to a new tube and fresh lysis buffer was added to the lysis tube and homogenization, incubation, and centrifugation was repeated with the supernatants being pooled. Next, nucleic acids were precipitated using 10 M ammonium acetate and isopropanol, with incubation on ice. The samples were centrifuged, supernatants aspirated, and the nucleic acid pellets were washed with ethanol and dried under vacuum. Pellets were dissolved in TE (Tris-EDTA) buffer. Finally, RNA and protein were removed, and the sample was purified. DNase-free RNase (10mg/mL) was added and the sample was incubated at 37^{o}C. Proteinase K and Buffer AL (from the QIAamp DNA Stool Mini Kit) were added and incubated at 70^{o}C. Ethanol was added and the sample was transferred to a QIAamp column and centrifuged. Flow through was discarded and Buffer AW1 and AW2 were added, with centrifugation and flow through being discarded each time. DNA was eluted using

Buffer AE. Quality was checked on a 0.8% agarose gel with 1 kb ladder, stained with ethidium bromide.

DNA Purification.--

Because the samples were extremely "dirty" with impurities that inhibit PCR amplification, such as humic acid, clay, DNA from indigenous organisms, metal ions or chelators (Menking et al. 1999), they were re-purified. Empty spin columns from Centri-Sep Kit were washed by centrifugation at high speed (13.2 rpm, 16.1 rcf) for 1.5 min three times with 0.3 mL TE each time. The gel was created with 0.8 mL of Sepharose 4B and allowed to settle for 1 min. It was then centrifuged as 2.9 rpm (0.8 rcf) for 2 min and washed three times with 0.5 mL TE by centrifuging 1.5 min at 2.9 rpm. During each centrifugation, the same orientation of the column was maintained to create a slant in the gel. In the middle of the gel, 20 µl of DNA was dispensed and column was placed into a 1.5 mL eppendorf tube. Tubes were centrifuged at 2.9 rpm for 2 min and purified DNA collected at bottom of tubes. DNA quality was checked on a 0.8% agarose gel with 1 kb ladder, stained with ethidium bromide.

Quantification and Dilution.--

Quantification of purified DNA samples was achieved using Quant-iT DNA Assay Kit, Broad Range (Molecular Probes, invitrogen detection technologies). A working solution (1:200 dilution) using 200x Quant-iT DNA BR reagent and Quant-iT DNA BR dilution buffer was made. In a 96 well microplate, 50 µl of working solution was dispensed. Triplicate λ DNA standards (from Quant-iT Kit) were made by adding 2.5 µl of standards (0, 5, 10, 20, 40, 60, 80, 100 ng/µl). Duplicate DNA samples were made by adding 2.5 µl of purified DNA. The microplate was mixed and centrifuged at 1,500 rpm and run on Real-Time PCR- Quantitative

Plate Read (Mx3000P). Using the quantities obtained, DNA samples were diluted with EB Buffer to equal 20 ng/µl.

PCR for Total Bacteria.--

A PCR was set up using 20 ng/µl of purified DNA and total bacteria primers GC-357f and 519r. For each sample, 98 µl of Master Mix (water, 10x PCR Buffer, 50mM $MgCl_2$, 3.36% BSA, 100 µM dNTP, 100 µM FW primer, 100 µM RV primer, and 5 U/µl Platinum Taq) and 2 µl of 20 ng/µl purified DNA sample. These were mixed, centrifuged, and ran on thermocycler program DGGE-V3 (Touch-down), modified from Kawai et al. 2002 (see Appendix B, Table 2 for modified program). A 1.5% agarose gel, stained with ethidium bromide, with a 100 bp ladder was run to check DNA quality.

Concentrate PCR Product.--

Because of the low quantity of DNA, the PCR product was concentrated 4-fold using 3 M (pH 5.2) 10 µl sodium acetate and 1 mL ethanol. Samples were mixed and incubated on ice for 30 min, then centrifuged on high for 15 min. The supernatant was removed via aspiration and the nucleic acid pellet was washed with 0.5 mL 70% ethanol, centrifuged on high for 15 min, and supernatant was removed again. Pellets were dried with a vacuum for 4 min and 25 µl TE was added to dissolve the pellet. DNA quality was checked on a 1.5% agarose gel with 100 bp ladder, stained with ethidium bromide.

DGGE for Total Bacteria.--

The microbial analysis was performed by Polymerase Chain Reaction and Denaturing Gradient Gel Electrophoresis (PCR-DGGE). This combined method is common to study the structure and genetic diversity of the microbial communities. Prior to performing DGGE, PCR primers that amplify the third hypervariable region (V3) within the gene encoding 16S ribosomal

RNA were used to generate a mixed population of PCR products representing all of the predominant species present in the soil sample (Yu and Morrison 2004*a*). After amplification, the DNA regions that are amplified now contain, in addition to each bacterium's DNA sequence, a 30 basepair GC clamp at one end. This hydrogen bonding of the GC basepairs within the clamp makes it resistant to denaturation, but the hypervariable region is not. Therefore, upon electrophoresis, the PCR products will migrate through a denaturing gradient of increasing concentrations of urea and formamide, to the point where only the GC clamp remains intact, at which point the PCR product stops (Myers et al. 1985). In other words, DGGE separates DNA segments of the same lengths based on the differences in GC content, and DNA molecules that differ even by only one nucleotide will have a different melting temperature. Once the gel was done running, the DNA was stained with a dye and visualized with UV light. Each sample resulted in a pattern of bands down the gel, which then were compared. Once a database was collected over three years, differences were recognized, allowing any changes to be monitored in soil microbial diversity over the experimental period.

The DGGE analysis was performed using a gradient of 45-58% (by mass) denaturants in acrylamide gels. The low gradient solution (45%) was made by combining with 8.8 mL of 6.5% acrylamide/ 0% denaturant with 7.2 mL 6.5% acrylamide/ 100% denaturant. The high gradient solution (58%) was made by combining 6.7 mL 6.5% acrylamide/ 0% denaturant with 9.3 mL 6.5% acrylamide/ 100% denaturant. Then 160 µl of 10% ammonium persulfate (APS) and 16 µl Tetramethylethylene-diamine (TEMED) were added and mixed together, and the gradient was poured. Once the gel was fully polymerized, 15 µl of each sample were loaded, along with 5 µl 100 bp ladder (to provide reference bands for gel normalization) and gels were run in 0.5x Tris-

Acetate-EDTA (TAE) at 60^{o}C at 82 volts for 16 hours. Gels were stained with 10,000x SYBR Green 1 nucleic acid gel stain for 30 min.

Dendrogram of Relatedness.--

Using Bionumerics (Applied Maths), trees of relatedness for the soil samples collected in different wetlands, at different conditions, in different seasons and years were constructed. Dice, a similarity coefficient that is band based with a dendrogram type of UPGMA, was used with 1.00% position tolerance for band comparison. Multiple gels had to be compared due to a large sample size of 81. Internal reference positions were assigned within lanes inside each DGGE gel image, allowing comparison within a gel. This reduces chance of error due to position of a sample on the gel. External reference positions were also assigned, which allowed comparison between gels.

These microbial data were compared with the findings and observations from the vegetation sampling and soil analyses. By doing so, a more holistic understanding of wetland disturbance and amelioration is forthcoming.

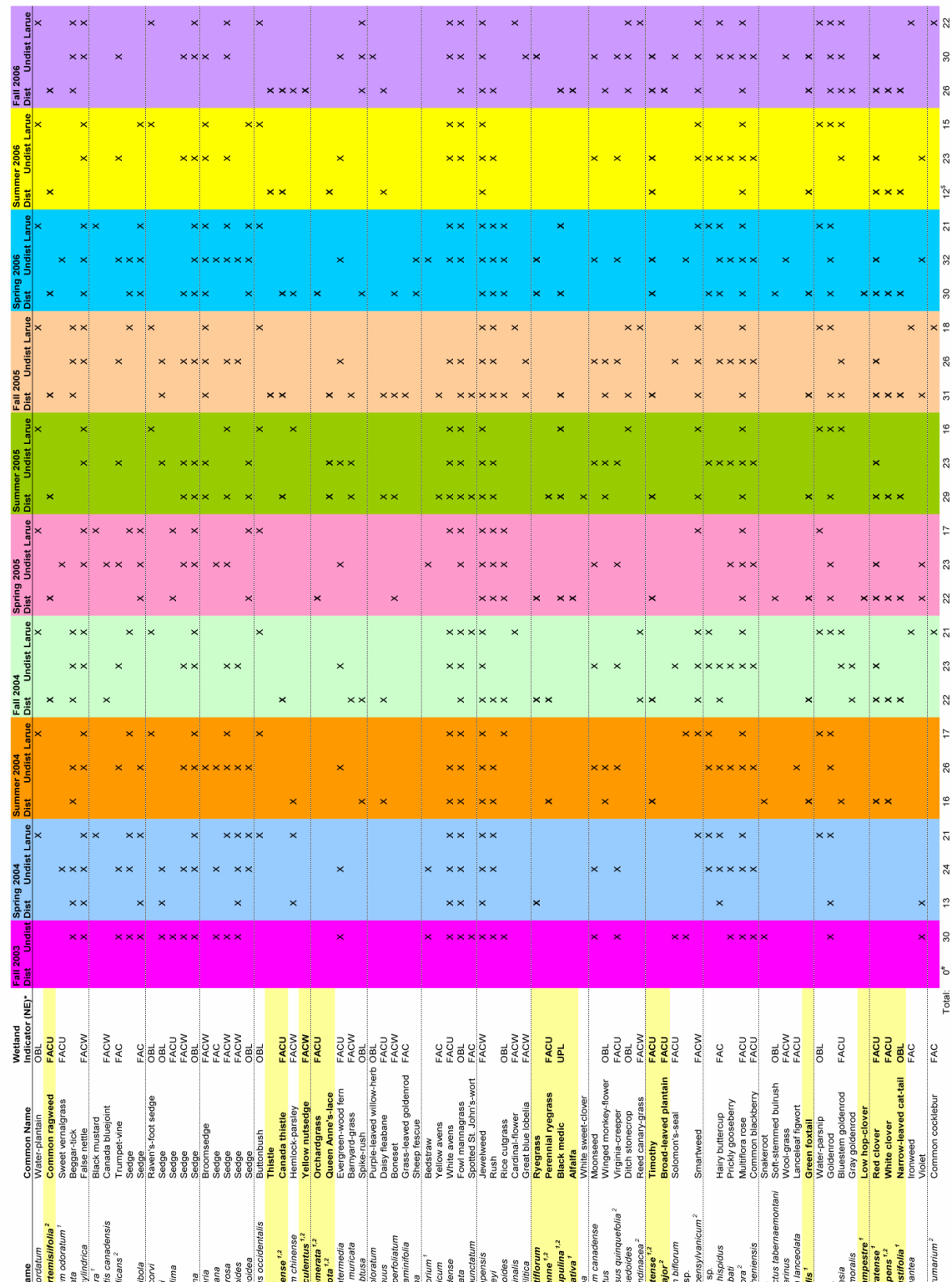
RESULTS

Vegetation Results

Construction of the pipeline was completed in Fall 2003 and a baseline vegetation survey of Disturbed and Undisturbed was taken immediately afterwards. Thus, in this baseline survey, Disturbed had zero species present while Undisturbed had 30 species present (Table 3). Total species increased in Disturbed with time and roughly equaled both Undisturbed and Larue by Fall 2004. In Summer 2006, Disturbed total species number decreased due to mowing of site prior to survey.

Highlighted species in Table 3 indicate species that first occurred in Disturbed and are considered introduced (US) and/or invasive and noxious (NE; USDA 2007). The majority of these highlighted species are either facultative upland or obligate upland species. It is also important to note that some species that first occurred in Disturbed are also present in Undisturbed by the end of the three year study period, such as *Trifolium pratense* (Red clover), *Setaria viridis* (Green foxtail), *Phleum pretense* (Timothy), and *Lolium multiflorum* (Ryegrass).

Table 3. Species present in wetlands for three years, plus baseline. Highlighted species first occurred in Disturbed and are considered introduced and/or invasive and noxious.



Scientific Name	Common Name	Wetland Indicator (NE)*	Fall 2003		Spring 2004		Summer 2004		Fall 2004		Spring 2005		Summer 2005		Fall 2005		Spring 2006		Summer 2006		Fall 2006
			Dist	Undist	Dist	Undist	Dist	Undist	Dist	Undist	Dist	Undist	Dist	Undist	Dist	Undist	Dist	Undist	Dist	Undist	Dist
Alisma subcordatum	Water-plantain	OBL																			
Ambrosia artemisiifolia [2]	**Common ragweed**	**FACU**																			
Anthoxanthum odoratum [1]	Sweet vernalgrass	FACU																			
Bidens vulgata	Beggar-tick																				
Boehmeria cylindrica	False nettle	FACW																			
Brassica nigra [1]	Black mustard																				
Calamagrostis canadensis	Canada bluejoint	FACW																			
Campsis radicans [2]	Trumpet-vine	FAC																			
Carex sp.	Sedge																				
Carex amphibola	Sedge	FAC																			
Carex crus-corvi	Raven's-foot sedge	OBL																			
Carex frankii	Sedge	OBL																			
Carex gracilima	Sedge	FACU																			
Carex grayi	Sedge	FACW																			
Carex lupulina	Sedge	OBL																			
Carex scoparia	Broomsedge	FACW																			
Carex shortiana	Sedge	FAC																			
Carex squarrosa	Sedge	FACW																			
Carex tribuloides	Sedge	FACW																			
Carex vulpinoidea	Sedge	OBL																			
Cephalanthus occidentalis	Buttonbush	OBL																			
***Cirsium* sp.**	**Thistle**	**FACU**																			
Cirsium arvense [1,2]	**Canada thistle**	**FACU**																			
Conioselinum chinense	Hemlock-parsley	FACW																			
Cyperus esculentus [1,2]	**Yellow nutsedge**	**FACW**																			
Dactylis glomerata [1,2]	**Orchardgrass**	**FACU**																			
Daucus carota [1,2]	**Queen Anne's-lace**	**FACU**																			
Dryopteris intermedia	Evergreen-wood fern	FACU																			
Echinochloa muricata	Barnyard-grass	FACW																			
Eleocharis obtusa	Spike-rush	OBL																			
Epilobium coloratum	Purple-leaved willow-herb	OBL																			
Erigeron annuus	Daisy fleabane	FACU																			
Eupatorium perfoliatum	Boneset	FACW																			
Euthamia graminifolia	Grass-leaved goldenrod	FAC																			
Festuca ovina	Sheep fescue																				
Galium tinctorium [1]	Bedstraw																				
Geum aleppicum	Yellow avens	FAC																			
Geum canadense	White avens	FACU																			
Glyceria striata	Fowl mannagrass	OBL																			
Hypericum punctatum	Spotted St. John's-wort	FACU																			
Impatiens capensis	Jewelweed	FACW																			
Juncus dudleyi	Rush																				
Leersia oryzoides	Rice cutgrass	OBL																			
Lobelia cardinalis	Cardinal-flower	FACW																			
Lobelia siphilitica	Great blue lobelia	FACW																			
Lolium multiflorum	**Ryegrass**																				
Lolium perenne [1,2]	**Perennial ryegrass**	**FACU**																			
Medicago lupulina [1,2]	**Black medic**	**UPL**																			
Medicago sativa [1]	**Alfalfa**																				
Melilotus alba	White sweet-clover																				
Menispermum canadense	Moonseed																				
Mimulus alatus	Winged monkey-flower	OBL																			
Parthenocissus quinquefolia [2]	Virginia-creeper	FACU																			
Penthorum sedoides	Ditch stonecrop	OBL																			
Phalaris arundinacea [2]	Reed canary-grass	FACW																			
Phleum pratense [1,2]	**Timothy**	**FACU**																			
Plantago major [2]	**Broad-leaved plantain**	**FACU**																			
Polygonatum biflorum	Solomon's-seal	FACU																			
Polygonum sp.																					
Polygonum pensylvanicum [2]	Smartweed	FACW																			
Ranunculus sp.																					
Ranunculus hispidus	Hairy buttercup	FAC																			
Ribes cynosbati	Prickly gooseberry																				
Rosa multiflora [2]	Multiflora rose	FACU																			
Rubus allegheniensis	Common blackberry	FACU																			
Sanicula sp.	Snakeroot																				
Schoenoplectus tabernaemontani	Soft-stemmed bulrush	OBL																			
Scirpus cyperinus	Wool-grass	FACW																			
Scrophularia lanceolata	Lanceleaf figwort	FACU																			
Setaria viridis [1]	**Green foxtail**																				
Sium suave	Water-parsnip	OBL																			
Soldago sp.	Goldenrod																				
Soldago caesia	Bluestem goldenrod	FACU																			
Soldago nemoralis	Gray goldenrod																				
Trifolium campestre [1]	**Low hop-clover**																				
Trifolium pratense [1]	**Red clover**	**FACU**																			
Trifolium repens [1,2]	**White clover**	**FACU**																			
Typha angustifolia [1]	**Narrow-leaved cat-tail**	**OBL**																			
Vernonia gigantea	Ironweed	FAC																			
Viola sp.	Violet																				
Xanthium strumarium [2]	Common cocklebur	FAC																			
Total:			0	30	13	24	21	16	26	17	22	23	21	22	23	17	29	23	16	31	26

[1]Introduced (U.S.)*
[2]Invasive & Noxious (NE)*

OBL =Obligate Wetland
FACW =Facultative Wetland
FAC =Facultative
FACU =Facultative Upland
UPL =Obligate Upland

[#]Vegetation survey taken immediately after completion of pipeline construction; thus, Disturbed Wetland has zero species present.

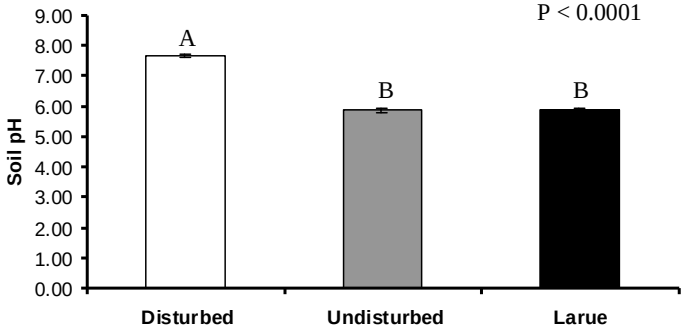
[3]Vegetation survey taken two weeks after Disturbed Wetland had been mowed.

*Information from United States Department of Agriculture, Natural Resources Conservation Service, http://plants.usda.gov/index.html

Soil Chemistry Results

Soil chemistry results were grouped by wetland (Disturbed, Undisturbed, and Larue) and statistical analyses using SAS: Mixed Procedure Method and Waller-Duncan Mean Separation were performed (n=27 for all soil chemistry analyses). Significant values for Disturbed as compared to Undisturbed and Larue were obtained for soil pH, P, B, Zn, Fe (lbs/acre), Cation Exchange Capacity, % Moisture, and % Organic Matter (Figure 3A and B). Larue was significantly higher than Disturbed and Undisturbed for NO_3 and S (lbs/acre) (Figure 4). NH_4-N (ppm) was significantly higher in Undisturbed than Disturbed and Larue (Figure 5). Disturbed was significantly different to Larue but not Undisturbed for Mn (lbs/acre) and Cu, K, Mg (lbs/acre), K, Ca, and Mg (% base saturation) resulted in all wetlands being significantly different to one another (Figure 6A and B). Ca (lbs/acre) was the only analyses that did not result in any significant differences between wetlands.

A.



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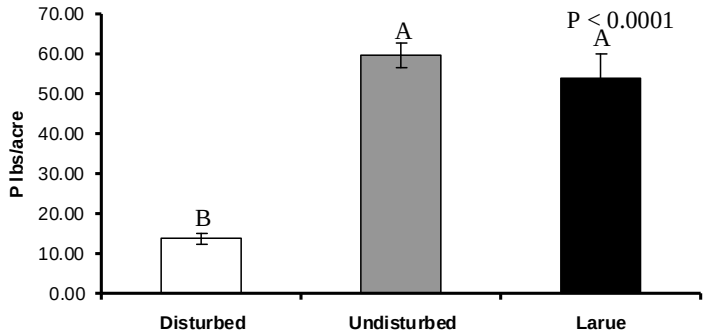
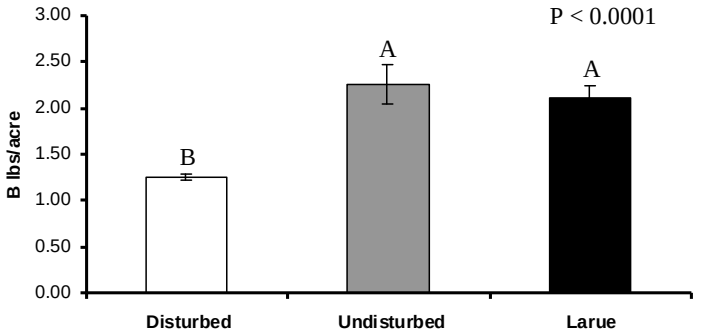
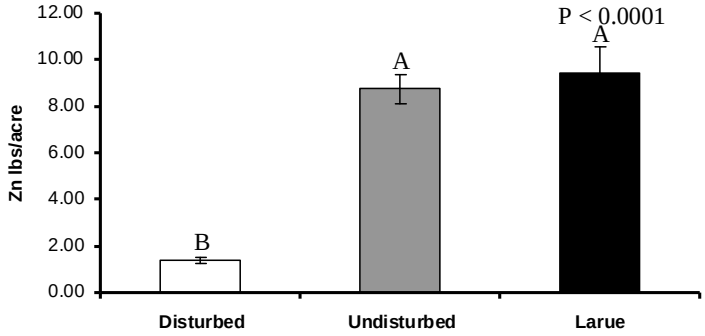
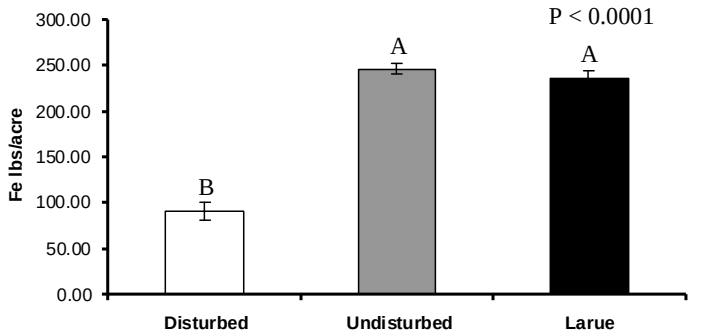
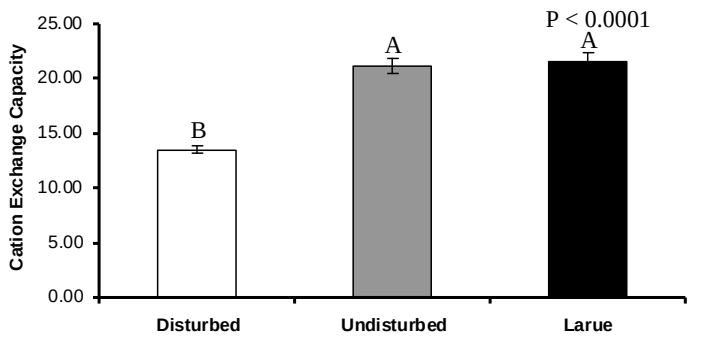
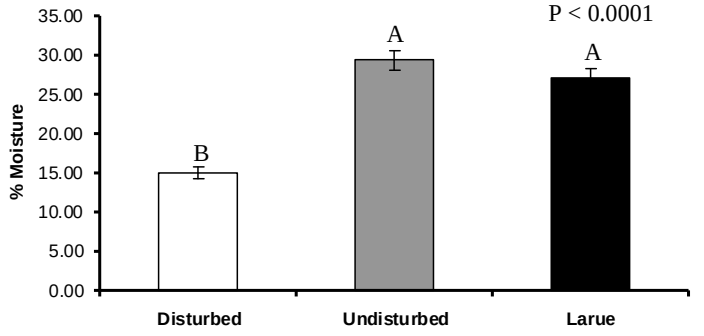



P < 0.0001
B lbs/acre
3.00
2.50
2.00
1.50
1.00
0.50
0.00
A
A
B
Disturbed
Undisturbed
Larue


P < 0.0001
Zn lbs/acre
12.00
10.00
8.00
6.00
4.00
2.00
0.00
A
A
B
Disturbed
Undisturbed
Larue


P < 0.0001
Fe lbs/acre
300.00
250.00
200.00
150.00
100.00
50.00
0.00
A
A
B
Disturbed
Undisturbed
Larue


P < 0.0001
Cation Exchange Capacity
25.00
20.00
15.00
10.00
5.00
0.00
A
A
B
Disturbed
Undisturbed
Larue


P < 0.0001
% Moisture
35.00
30.00
25.00
20.00
15.00
10.00
5.00
0.00
A
A
B
Disturbed
Undisturbed
Larue

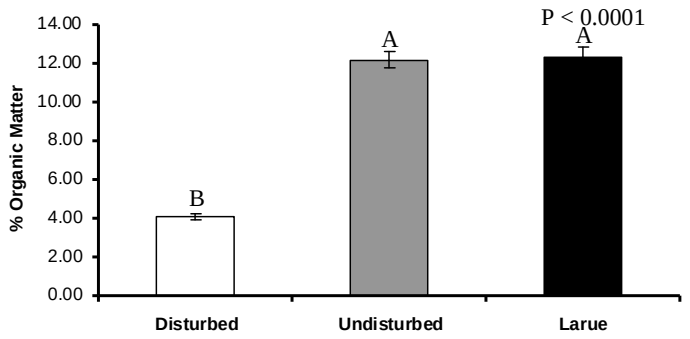


Figure 3A. Disturbed is significantly higher than Undisturbed and Larue. B. Disturbed is significantly lower than Undisturbed and Larue.

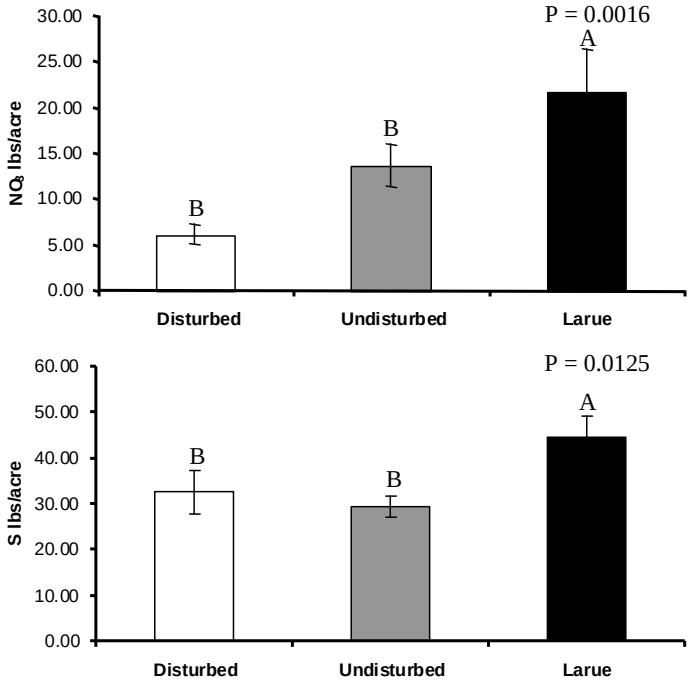


Figure 4. Larue is significantly higher than Disturbed and Undisturbed.

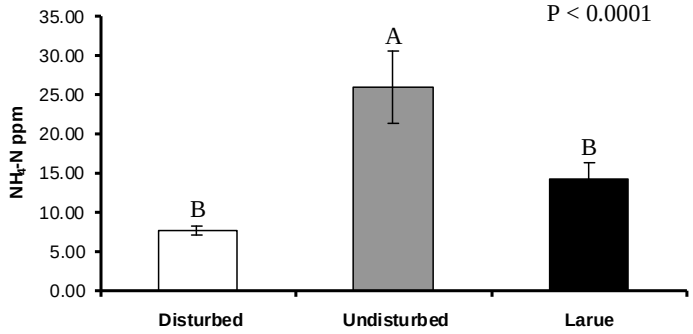
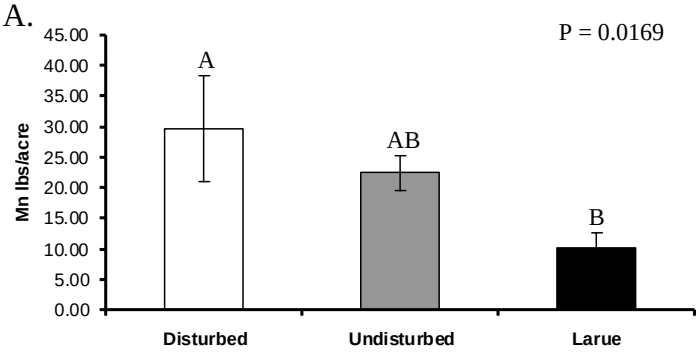
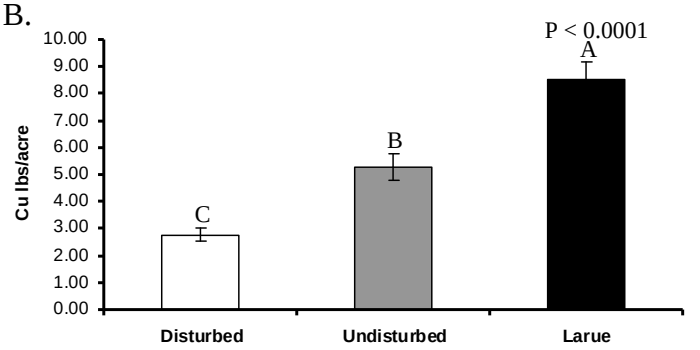


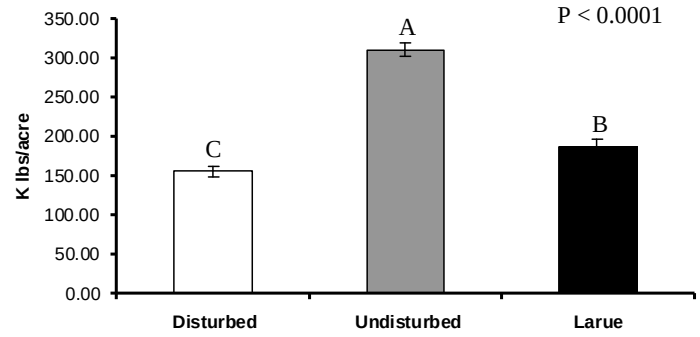
Figure 5. Undisturbed is significantly higher than Disturbed and Larue.

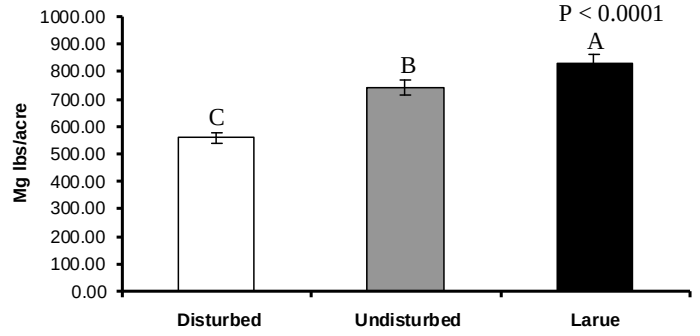
A.

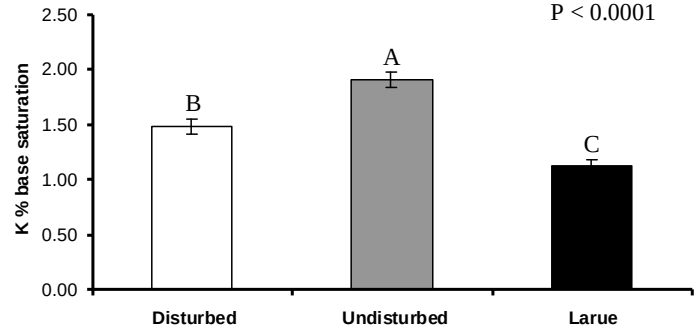

P = 0.0169
Mn lbs/acre
45.00
40.00
35.00
30.00
25.00
20.00
15.00
10.00
5.00
0.00
A
AB
B
Disturbed
Undisturbed
Larue

B.


P < 0.0001
Cu lbs/acre
10.00
9.00
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
C
B
A
Disturbed
Undisturbed
Larue


P < 0.0001
K lbs/acre
350.00
300.00
250.00
200.00
150.00
100.00
50.00
0.00
C
A
B
Disturbed
Undisturbed
Larue


P < 0.0001
Mg lbs/acre
1000.00
900.00
800.00
700.00
600.00
500.00
400.00
300.00
200.00
100.00
0.00
C
B
A
Disturbed
Undisturbed
Larue


P < 0.0001
K % base saturation
2.50
2.00
1.50
1.00
0.50
0.00
B
A
C
Disturbed
Undisturbed
Larue

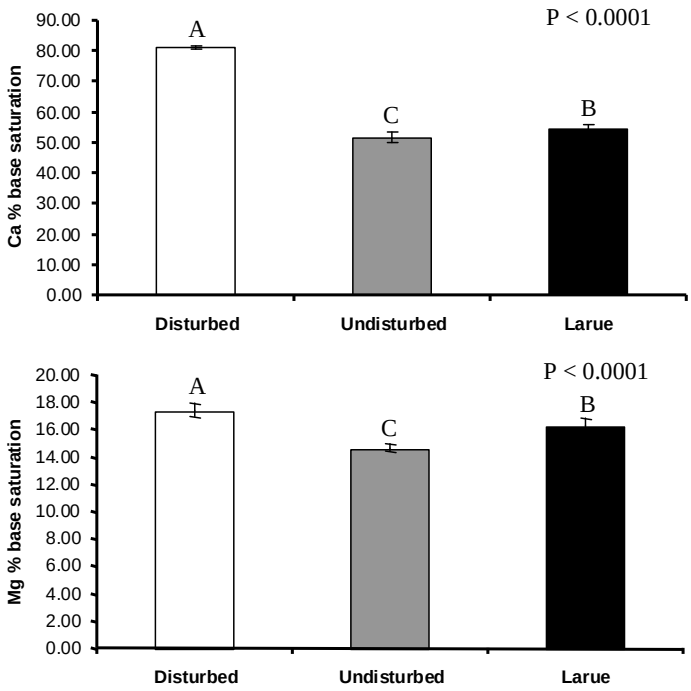


Figure 6A. Disturbed is significantly different to Larue but not Undisturbed. B. All three wetlands are significantly different to each other.

Percent Moisture Results

Soil moisture is lower in Disturbed soil samples as compared to Undisturbed and Larue (Figure 7). When DGGE gel images are viewed with the moisture graph, it is apparent that there is a correlation between microbial diversity and percent moisture. Disturbed samples have lower moisture and lower microbial diversity, as indicated by fewer bands on the DGGE gel images.

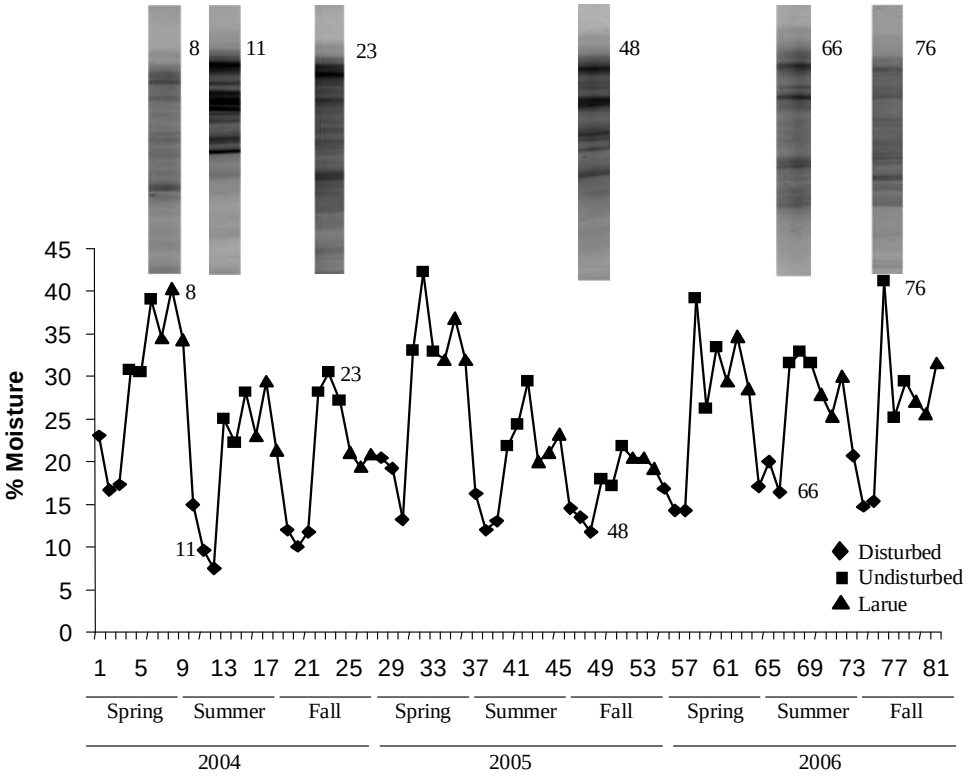


Figure 7. Soil moisture in samples from Disturbed, Undisturbed, and Larue. Percent moisture is lower in Disturbed samples as compared to Undisturbed and Larue. DGGE gel images of selected samples indicate that microbial activity is dependent on moisture as there is lower diversity (fewer bands) in the Disturbed samples.

Soil Permeability Results

Soil permeability was the lowest in the Disturbed wetland, with a permeability of only 1.65×10^{-8} cm/sec. Undisturbed had a permeability of 6.84×10^{-8} cm/sec and the value for Larue was 1.06×10^{-4} cm/sec (n=1) (Figure 8).

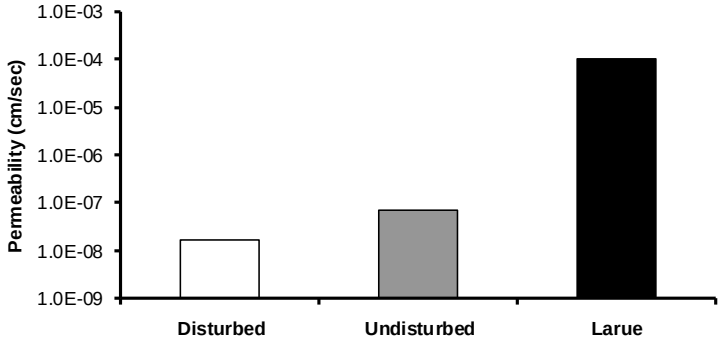


Figure 8. Soil permeability was the lowest in Disturbed. The Disturbed value should be closer in value to Undisturbed as the two sites are adjacent to each other; thus, the pipeline construction and disturbance decreased permeability.

Soil Microbial Results

Soil microbial diversity was explored using PCR and DGGE, with the DGGE images analyzed and compared with Bionumerics computer program. First, all 81 soil samples were compared together (Figure 9) in a dendrogram (tree of relatedness). Near the top of the image, there is a clear banding pattern where almost all of the samples contain a similar band or bands. There are also smaller similar banding patterns within the gel. Due to the complexity of the figure, it is difficult to distinguish other relationships, so the images were further divided for more detailed analyses.

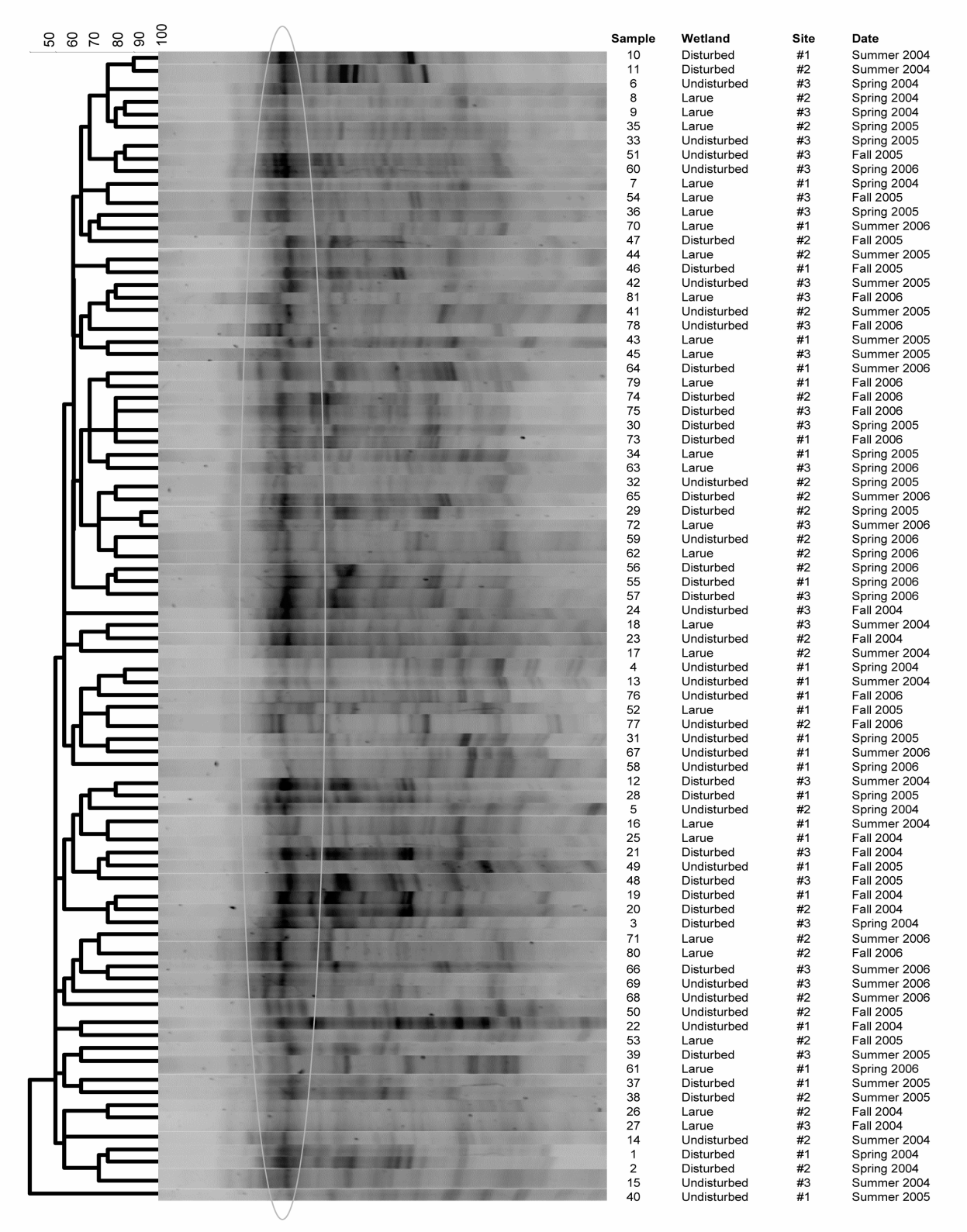
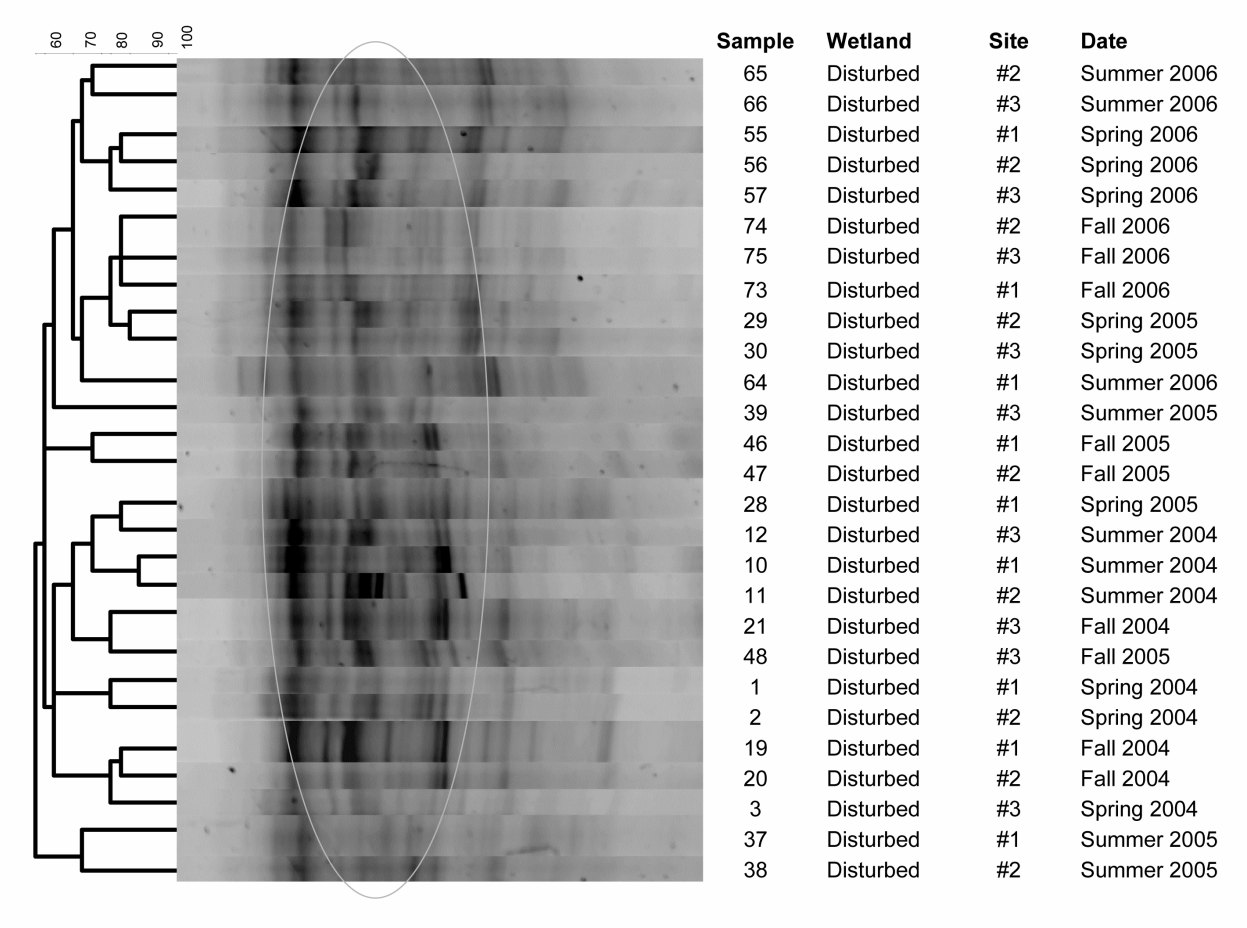


Figure 9. Microbial comparison for all 81 samples. There is a clear banding pattern near the top of the gel (circle) and smaller patterns are visible within the gel.

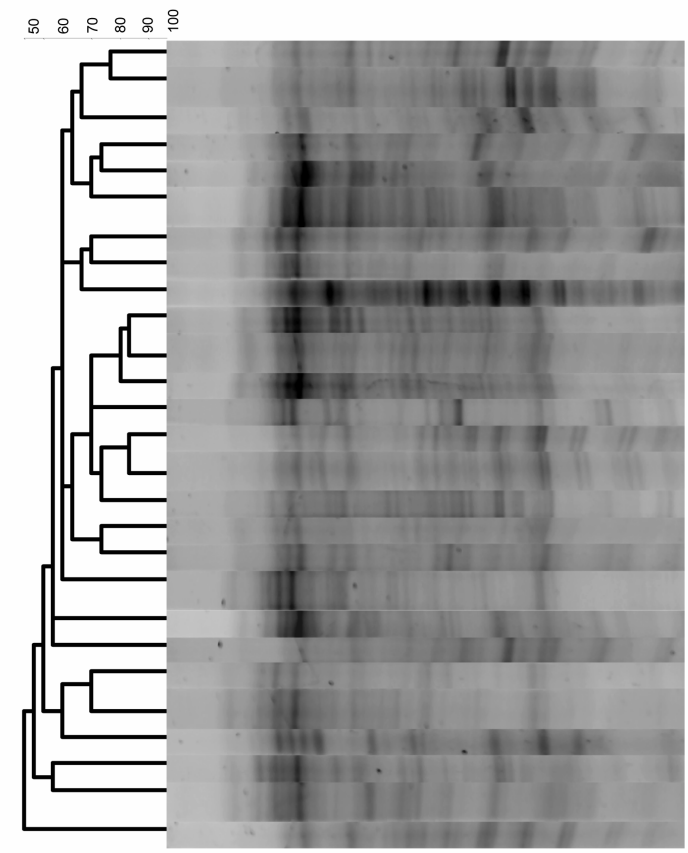
Next, dendrograms were constructed for each wetland: Disturbed, Undisturbed, and Larue (Figure 10 A-C). Grouping by wetland, it is easy to see that each wetland is more similar to itself than to each other; thus, the main influence on the microbial community is the wetland itself. Undisturbed and Larue are more similar to each other than Disturbed is to either one. The Disturbed wetland appears to have less overall diversity with more banding concentrated closer to the top of the gel.

A.



Sample	Wetland	Site	Date
65	Disturbed	#2	Summer 2006
66	Disturbed	#3	Summer 2006
55	Disturbed	#1	Spring 2006
56	Disturbed	#2	Spring 2006
57	Disturbed	#3	Spring 2006
74	Disturbed	#2	Fall 2006
75	Disturbed	#3	Fall 2006
73	Disturbed	#1	Fall 2006
29	Disturbed	#2	Spring 2005
30	Disturbed	#3	Spring 2005
64	Disturbed	#1	Summer 2006
39	Disturbed	#3	Summer 2005
46	Disturbed	#1	Fall 2005
47	Disturbed	#2	Fall 2005
28	Disturbed	#1	Spring 2005
12	Disturbed	#3	Summer 2004
10	Disturbed	#1	Summer 2004
11	Disturbed	#2	Summer 2004
21	Disturbed	#3	Fall 2004
48	Disturbed	#3	Fall 2005
1	Disturbed	#1	Spring 2004
2	Disturbed	#2	Spring 2004
19	Disturbed	#1	Fall 2004
20	Disturbed	#2	Fall 2004
3	Disturbed	#3	Spring 2004
37	Disturbed	#1	Summer 2005
38	Disturbed	#2	Summer 2005

B.



Sample	Wetland	Site	Date
31	Undisturbed	#1	Spring 2005
67	Undisturbed	#1	Summer 2006
49	Undisturbed	#1	Fall 2005
41	Undisturbed	#2	Summer 2005
42	Undisturbed	#3	Summer 2005
23	Undisturbed	#2	Fall 2004
5	Undisturbed	#2	Spring 2004
6	Undisturbed	#3	Spring 2004
22	Undisturbed	#1	Fall 2004
51	Undisturbed	#3	Fall 2005
33	Undisturbed	#3	Spring 2005
60	Undisturbed	#3	Spring 2006
77	Undisturbed	#2	Fall 2006
4	Undisturbed	#1	Spring 2004
13	Undisturbed	#1	Summer 2004
76	Undisturbed	#1	Fall 2006
32	Undisturbed	#2	Spring 2005
59	Undisturbed	#2	Spring 2006
78	Undisturbed	#3	Fall 2006
24	Undisturbed	#3	Fall 2004
58	Undisturbed	#1	Spring 2006
68	Undisturbed	#2	Summer 2006
69	Undisturbed	#3	Summer 2006
50	Undisturbed	#2	Fall 2005
14	Undisturbed	#2	Summer 2004
15	Undisturbed	#3	Summer 2004
40	Undisturbed	#1	Summer 2005

C.

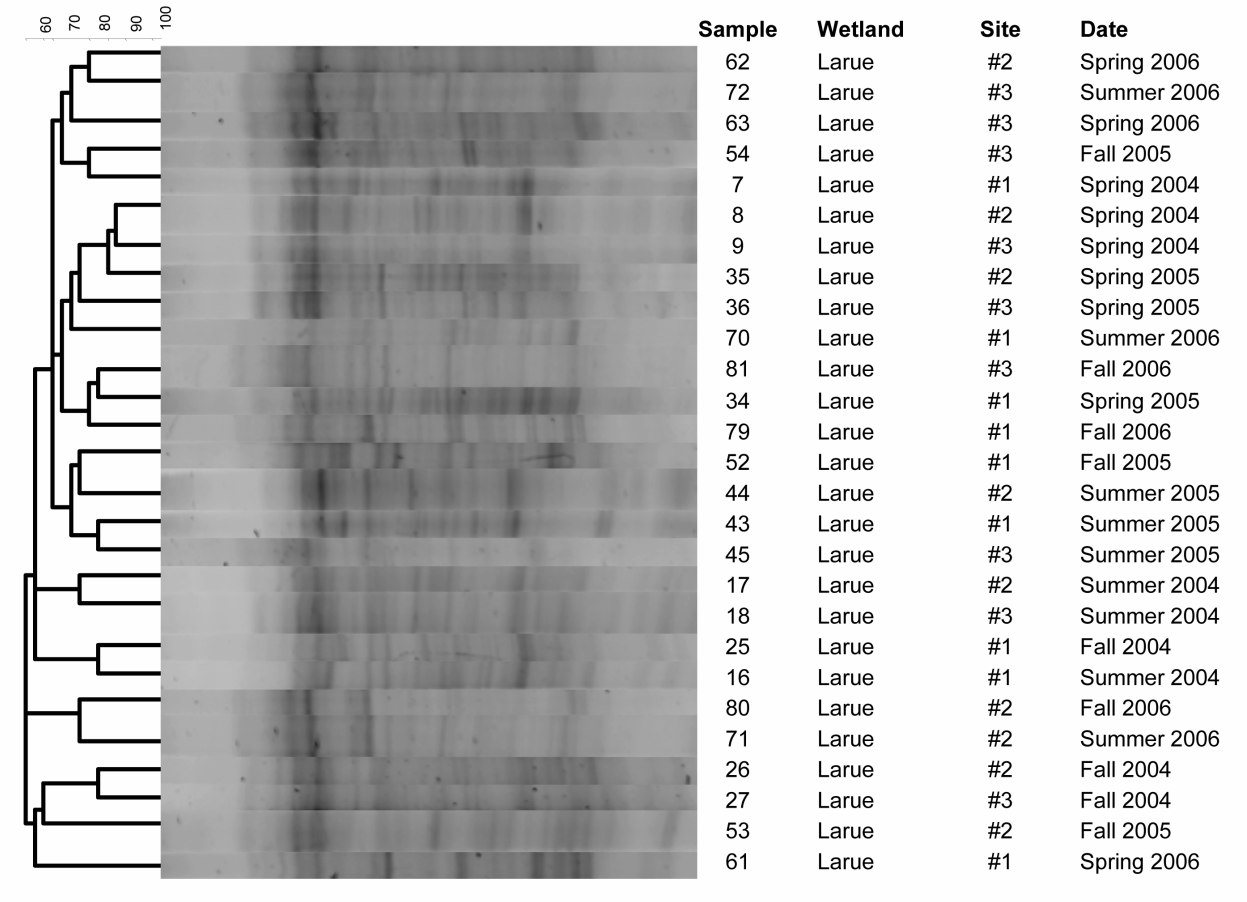
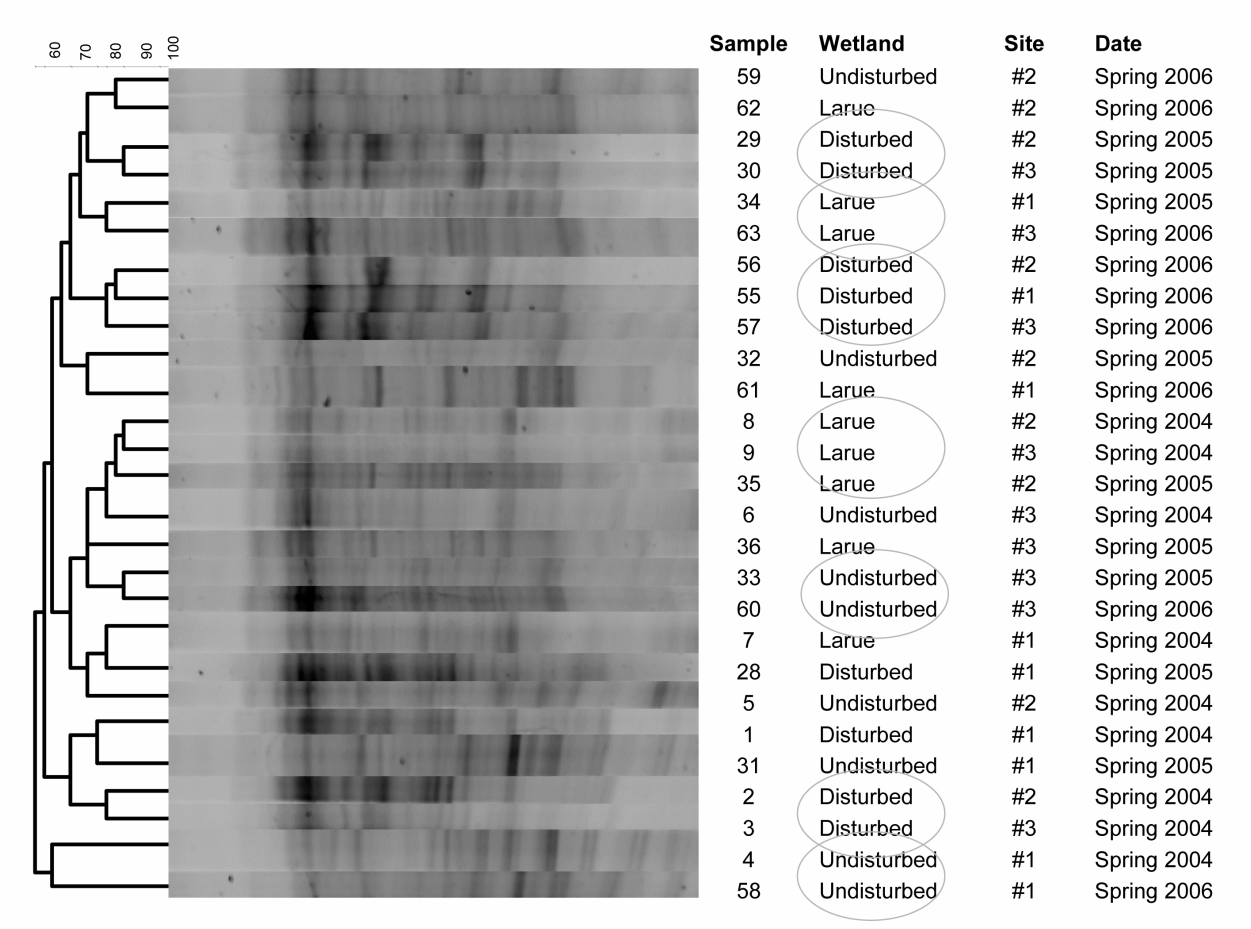


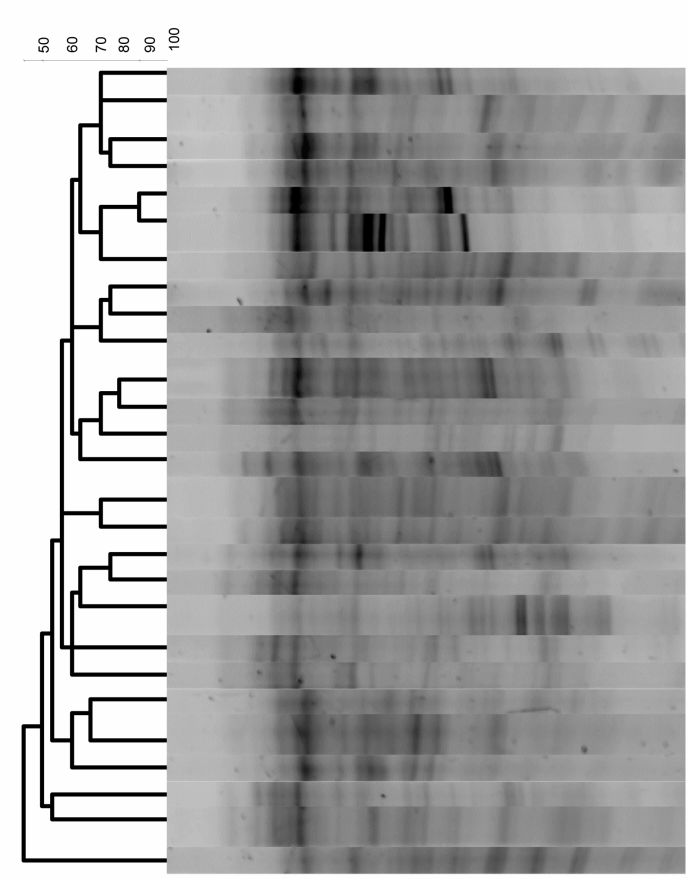
Figure 10. A. The Disturbed banding pattern is more similar to itself than Undisturbed or Larue. Notice that the majority of bands are located closer to the top of the gel (circle). B. The Undisturbed banding pattern is more similar to itself than Disturbed or Larue. C. The Larue banding pattern is more similar to itself than Disturbed or Undisturbed.

Dendrograms were created comparing seasons: Spring (Figure 11 A), Summer (Figure 11 B), and Fall (Figure 11 C). There is some blocking within seasons via wetland.

A.


60
70
80
90
100
Sample Wetland Site Date
59 Undisturbed #2 Spring 2006
62 Larue #2 Spring 2006
29 Disturbed #2 Spring 2005
30 Disturbed #3 Spring 2005
34 Larue #1 Spring 2005
63 Larue #3 Spring 2006
56 Disturbed #2 Spring 2006
55 Disturbed #1 Spring 2006
57 Disturbed #3 Spring 2006
32 Undisturbed #2 Spring 2005
61 Larue #1 Spring 2006
8 Larue #2 Spring 2004
9 Larue #3 Spring 2004
35 Larue #2 Spring 2005
6 Undisturbed #3 Spring 2004
36 Larue #3 Spring 2005
33 Undisturbed #3 Spring 2005
60 Undisturbed #3 Spring 2006
7 Larue #1 Spring 2004
28 Disturbed #1 Spring 2005
5 Undisturbed #2 Spring 2004
1 Disturbed #1 Spring 2004
31 Undisturbed #1 Spring 2005
2 Disturbed #2 Spring 2004
3 Disturbed #3 Spring 2004
4 Undisturbed #1 Spring 2004
58 Undisturbed #1 Spring 2006

B.



Sample	Wetland	Site	Date
12	Disturbed	#3	Summer 2004
41	Undisturbed	#2	Summer 2005
42	Undisturbed	#3	Summer 2005
44	Larue	#2	Summer 2005
10	Disturbed	#1	Summer 2004
11	Disturbed	#2	Summer 2004
16	Larue	#1	Summer 2004
43	Larue	#1	Summer 2005
45	Larue	#3	Summer 2005
13	Undisturbed	#1	Summer 2004
65	Disturbed	#2	Summer 2006
72	Larue	#3	Summer 2006
70	Larue	#1	Summer 2006
64	Disturbed	#1	Summer 2006
17	Larue	#2	Summer 2004
18	Larue	#3	Summer 2004
66	Disturbed	#3	Summer 2006
69	Undisturbed	#3	Summer 2006
67	Undisturbed	#1	Summer 2006
68	Undisturbed	#2	Summer 2006
71	Larue	#2	Summer 2006
37	Disturbed	#1	Summer 2005
38	Disturbed	#2	Summer 2005
39	Disturbed	#3	Summer 2005
14	Undisturbed	#2	Summer 2004
15	Undisturbed	#3	Summer 2004
40	Undisturbed	#1	Summer 2005

C.

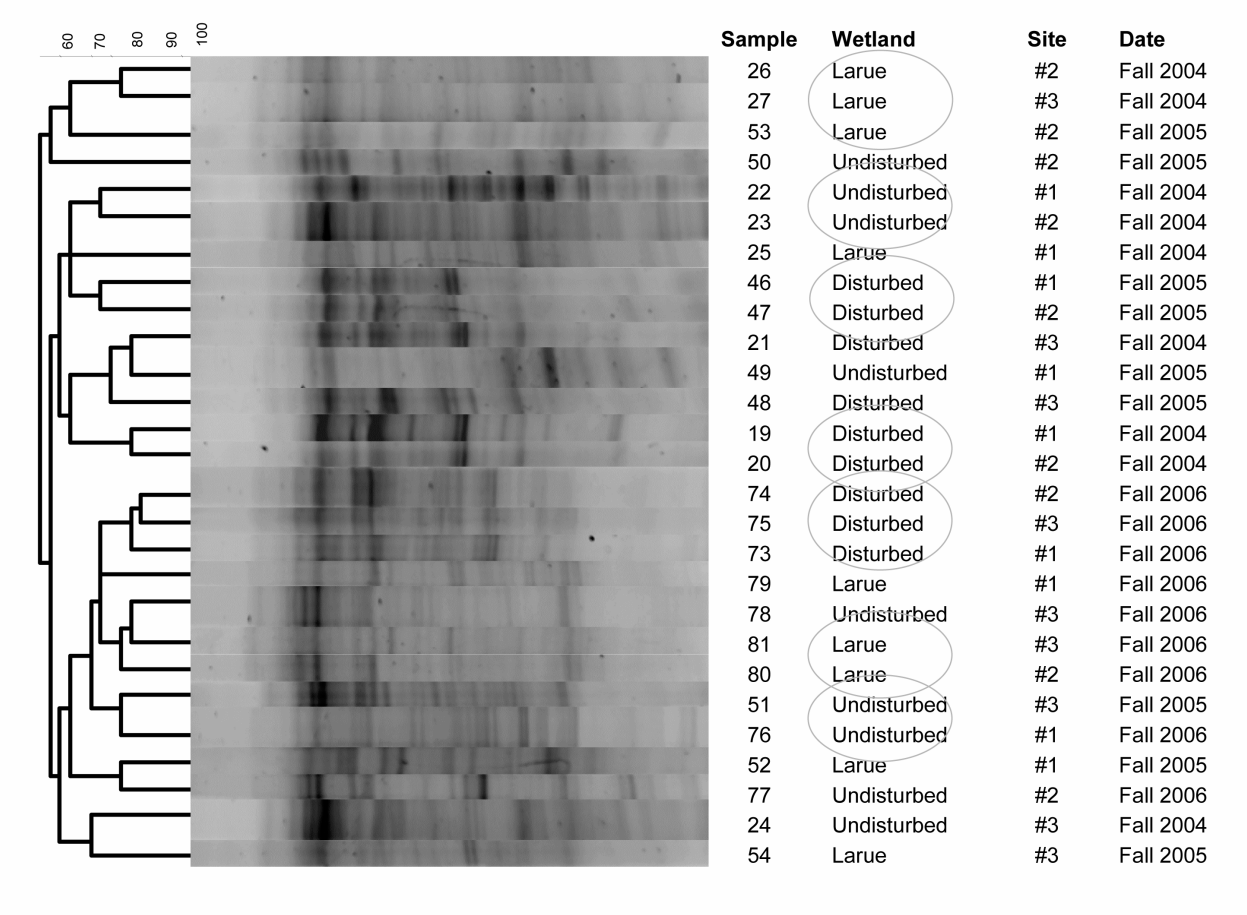


Figure 11A-C. In dendrograms separated by season, some blocking via wetland can be seen, indicating that wetland has the most effect on microbial communities.

Dendrograms were also created by site: #1- wet (Appendix C, Figure 12 A), #2- semi-wet (Appendix C, Figure 12 B), and #3- dry (Appendix C, Figure 12 C); and by year: 2004 (Appendix C, Figure 13 A), 2005 (Appendix C, Figure 13 B), and 2006 (Appendix C, Figure 13 C). These dendrograms had similar results as dendrograms separated by season. In addition, in #1- wet (Figure 12 A), there is slightly higher diversity, indicating the water is needed for microbial growth. The year dendrograms are similar; therefore, the wetlands are relatively stable and the microbial community after disturbance is retained. Again, this indicates that wetland has the greatest influence on microbial community structure.

Using the Bionumerics program to calculate number of peaks (bands) on gel images and the Shannon Diversity Index formula, a diversity value for each sample was obtained. Values were averaged for each wetland (n=27) (Figure 14). Figure 14 actually contradicts previous results of Disturbed having lowest diversity; however, the diversity values are very similar and error bars indicate no difference between wetlands.

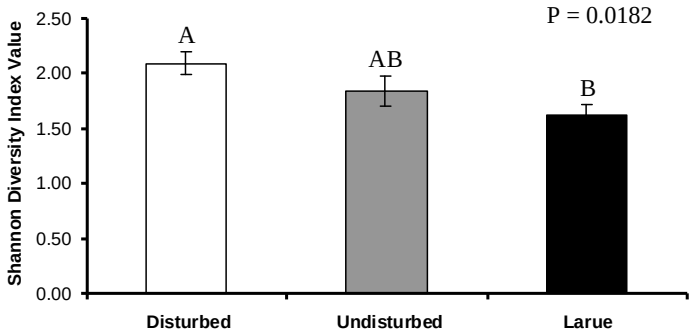


Figure 14. Diversity values for each wetland were obtained using data from the Bionumerics program and the Shannon Diversity Index formula. Close values and error bars indicate no significant difference in diversity between wetlands.

DISCUSSION

Disturbance causes significant changes to many aspects of a wetland ecosystem. Each of these aspects can be analyzed separately, but in order to obtain a more complete picture of the effects of disturbance, the relationships between aspects must be considered. The aim of this research is to integrate multiple ideas and concepts to create a more holistic understanding of disturbance.

After the construction of the pipeline was completed in Fall 2003, the total number of species in Disturbed steadily increased until it roughly equaled Undisturbed and Larue. However, this increase in species richness was primarily in species that are introduced and/or invasive and noxious. Some of these species have also spread to the Undisturbed wetland. Several further disturbances were created by the pipeline company; during construction, the topsoil was not segregated, the Disturbed wetland was seeded and fertilized after construction, and the Disturbed wetland was mowed once during this study period in Summer 2006. The seed mixture was probably legume-based, with most of the species being facultative upland or obligate upland species. This can explain the large increase in legumes and upland species occurring so quickly after completion of construction. The Disturbed wetland had the lowest permeability (Figure 8), which may have been a direct result from the process of construction and compaction by equipment. This decreased permeability creates a less desirable environment for growth as it hinders soil water and airflow, mineral flow, and root extension (Singer and Munns 2006).

Vegetation and soil chemistry affect each other; for example, with the increase in legumes, there would be a subsequent increase in nitrogen-fixing bacteria, which may cause an increase in nitrogen present in the soil. The application of fertilizer may have caused the

increase in pH for Disturbed (Figure 3A). The % organic matter (Figure 3B) in Disturbed was remarkably lower than in Undisturbed and Larue, which is probably a result of not segregating the topsoil. Changes in the soil chemistry, either higher or lower, will affect the vegetation and microbial communities.

The microbial analysis illustrates a common band in almost all of the samples (Figure 9), which would presumably be a widespread and frequently occurring bacterial species. Upon separating the DGGE images by wetland, it can be seen that the wetlands are more similar to themselves than each other- indicating that the main influence to changes in microbial communities is the wetland itself, and its inherent characteristic (disturbed or undisturbed). The Undisturbed and Larue wetlands have a more similar profile to each other than the Disturbed wetland, further illustrating that the disturbance of the pipeline construction has directly affected the microbial community. It is interesting that Undisturbed and Larue are more similar, especially considering that they are 0.4 km apart, whereas Undisturbed and Disturbed are adjacent to each other. Other comparisons also have banding patterns according to wetland.

If one presumes each band seen on DGGE gels indicates a different species, differences in overall diversity might be considered by comparing the number of bands: with fewer bands suggestive of there being less diversity. The computational analyses suggested the Disturbed wetland actually has a greater number of bands, i.e. suggestive of there being more diversity in this site. However, visual appraisal of the respective DGGE profiles provides a greater awareness. Most notable is that the banding profiles for virtually all of the samples from the Disturbed site migrate within a relatively narrow, upper range of the denaturing gradients. Bands located closer to the top of the gel should have a higher AT content and/or be shorter in length. Conversely, the profiles produced from the Undisturbed and Larue sites were more diverse, with

respect to the presence of bands throughout the gradient. Accordingly, these bands would represent bacterial species with V3 regions that are (relatively) more GC-rich and/or longer than those present in microbes from the Disturbed site. In other words, the term "diversity" could be applied in different contexts, and the computational and visual analyses might lead to different conclusions. To resolve this dilemma, further research, which recover the DNA bands from the gels and then re-amplifies them by PCR for DNA sequencing, would be useful. This would allow the prominent bands to be "assigned" to key bacterial phyla, and conclusively identify the range of microbes resident in each sample and site (Burr et al. 2006).

Over the study period of three years, there is little difference across years, which suggests that the microbial communities are relatively stable, even in the Disturbed wetland. This means that once a microbial community is disturbed, it will retain the effects of that disturbance, at least for some time period.

When dendrograms were created comparing site conditions (wet, semi-wet, dry), #1- wet (Appendix C, Figure 12A) had higher diversity, showing that with an increase in water, there was an increase in diversity. This conclusion is supported by the result of lower diversity and lower percent moisture in the Disturbed wetland (Figure 7). However, it is important to note that the designations of #1- wet, #2- semi-wet, and #3- dry were completely based on the individual wetlands; for example, Disturbed #1-wet site was much drier than the #1 sites for Undisturbed and Larue due to Disturbed having no standing water after pipeline construction.

The diversity value (Figure 14) is actually highest for Disturbed, which contradicts the conclusions described previously. However, the error bars indicate that there is no difference between wetlands. Furthermore, the limitations of the Bionumerics computer program must be taken into account. The computer program designates whether a band is present by position

tolerance settings. For this analysis, the setting was at the commonly used and accepted 1.00% position tolerance. Depending on the total amount of bands and circumstance of the study, the position tolerance can be adjusted. Soil DNA samples have extremely high diversity; perhaps with a more discriminating position tolerance setting, the computer program would yield a better reflection of band number. Examining the dendrograms visually, it is apparent that Disturbed has lower diversity than Undisturbed and Larue.

In conclusion, disturbance results in numerous alterations in wetland ecosystems, including changes, either directly or indirectly, in vegetation composition, soil chemical make-up, moisture, permeability, and microbial community structure.

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APPENDIX A

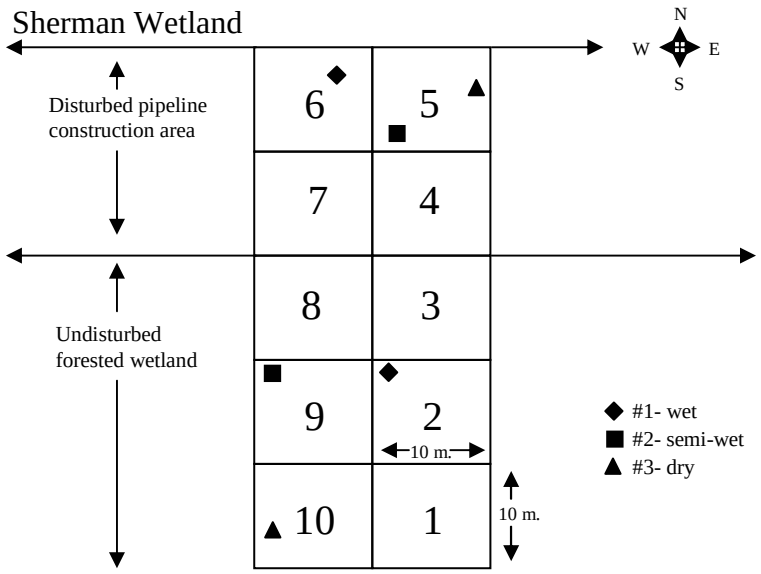


Figure 1. Diagram of the Sherman wetland, located in central Ohio, Pickaway Co., which houses both the Disturbed and Undisturbed wetland areas.

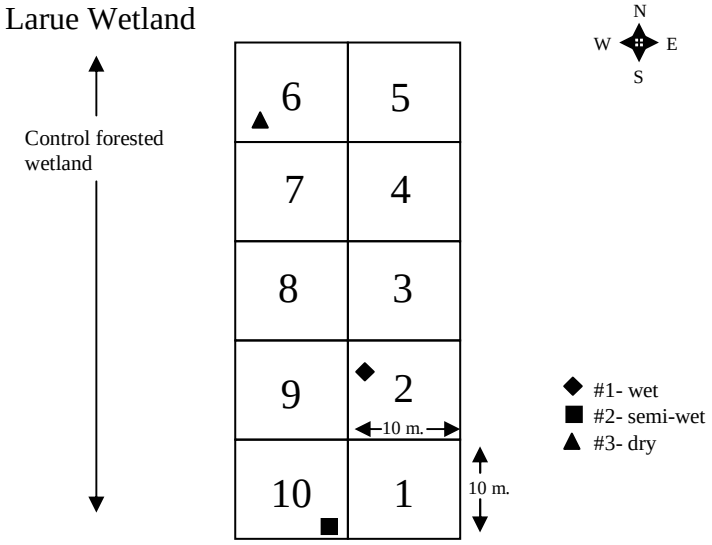


Figure 2. Diagram of the Larue wetland, located in central Ohio, Pickaway Co., which serves as the control wetland. The Sherman wetland and the Larue wetland are 0.4 km apart.

Table 1. Table of soil samples collected for three years in each wetland site. Each sample was assigned a specific sample number. The site refers to soil condition; #1 is wet, #2 is semi-wet, and #3 is dry. Sampling locations were marked so as to sample in the same area each time.

Sample	Wetland	Site	Date
1	Dist	#1	
2	Dist	#2	
3	Dist	#3	
4	Undist	#1	
5	Undist	#2	**Spring 2004**
6	Undist	#3	**5/29/04**
7	Larue	#1	
8	Larue	#2	
9	Larue	#3	
10	Dist	#1	
11	Dist	#2	
12	Dist	#3	
13	Undist	#1	
14	Undist	#2	**Summer 2004**
15	Undist	#3	**7/16/04**
16	Larue	#1	
17	Larue	#2	
18	Larue	#3	
19	Dist	#1	
20	Dist	#2	
21	Dist	#3	
22	Undist	#1	
23	Undist	#2	**Fall 2004**
24	Undist	#3	**9/5/04**
25	Larue	#1	
26	Larue	#2	
27	Larue	#3	
28	Dist	#1	
29	Dist	#2	
30	Dist	#3	
31	Undist	#1	
32	Undist	#2	**Spring 2005**
33	Undist	#3	**5/30/05**
34	Larue	#1	
35	Larue	#2	
36	Larue	#3	
37	Dist	#1	
38	Dist	#2	
39	Dist	#3	
40	Undist	#1	
41	Undist	#2	**Summer 2005**
42	Undist	#3	**7/17/05**
43	Larue	#1	
44	Larue	#2	
45	Larue	#3	

Table 1. Continued.

Sample	Wetland	Site	Date
46	Dist	#1	
47	Dist	#2	
48	Dist	#3	
49	Undist	#1	
50	Undist	#2	**Fall 2005**
51	Undist	#3	**9/5/05**
52	Larue	#1	
53	Larue	#2	
54	Larue	#3	
55	Dist	#1	
56	Dist	#2	
57	Dist	#3	
58	Undist	#1	
59	Undist	#2	**Spring 2006**
60	Undist	#3	**5/30/06**
61	Larue	#1	
62	Larue	#2	
63	Larue	#3	
64	Dist	#1	
65	Dist	#2	
66	Dist	#3	
67	Undist	#1	
68	Undist	#2	**Summer 2006**
69	Undist	#3	**7/15/06**
70	Larue	#1	
71	Larue	#2	
72	Larue	#3	
73	Dist	#1	
74	Dist	#2	
75	Dist	#3	
76	Undist	#1	
77	Undist	#2	**Fall 2006**
78	Undist	#3	**9/4/06**
79	Larue	#1	
80	Larue	#2	
81	Larue	#3	

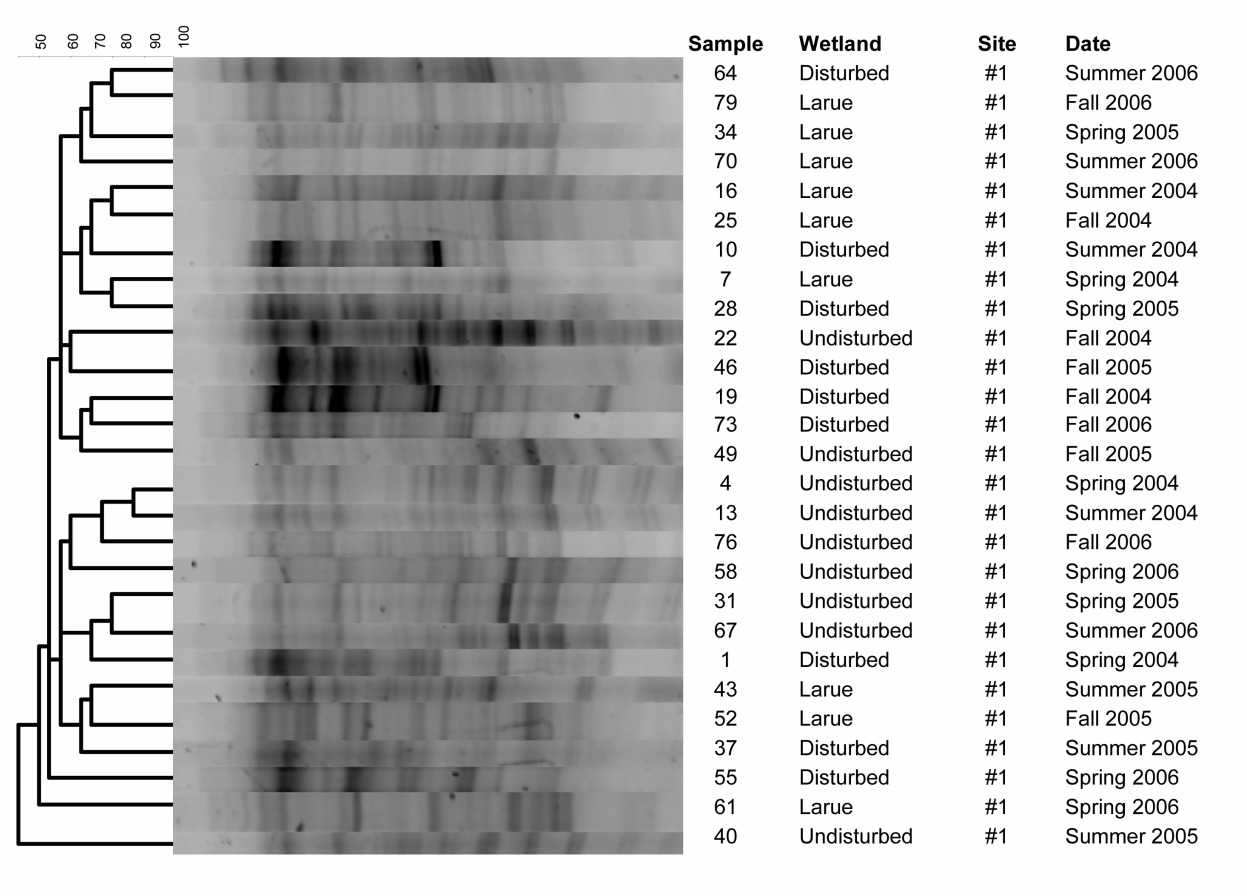
APPENDIX B

Table 2. Thermocycler program DGGE-V3 (Touch-down) for DNA amplification, modified from Kawai et al. 2002.

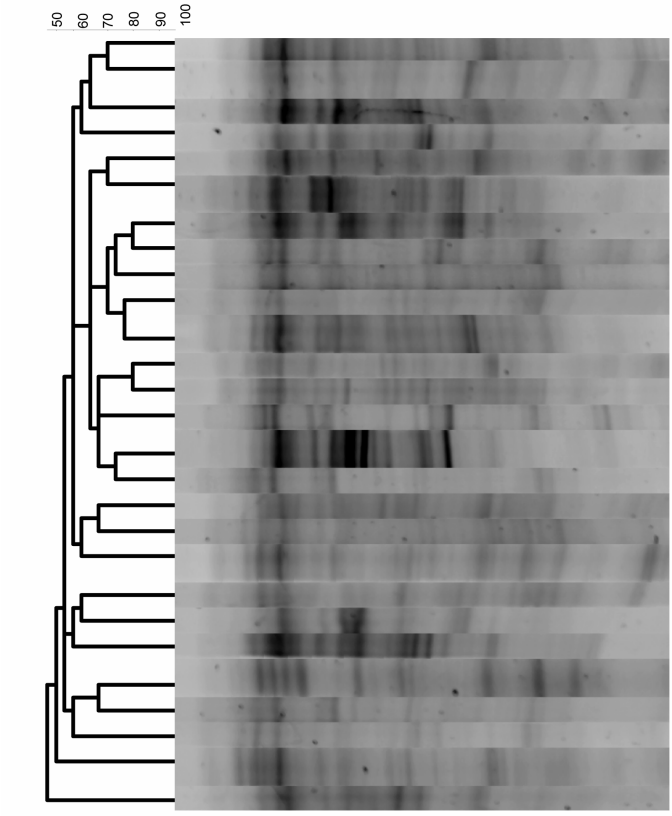
Step	Degrees	Time
1.	90^{o}	---
2.	94^{o}	4 min
3.	94^{o}	30 sec
4.	61^{o}	30 sec, decrease 0.5^{o} /cycle
5.	72^{o}	30 sec
6.	10x to step 3	
7.	94^{o}	30 sec
8.	56^{o}	30 sec
9.	72^{o}	30 sec
10.	25x to step 7	
11.	72^{o}	30 min
12.	4^{o}	---
13.	End	

APPENDIX C

A.

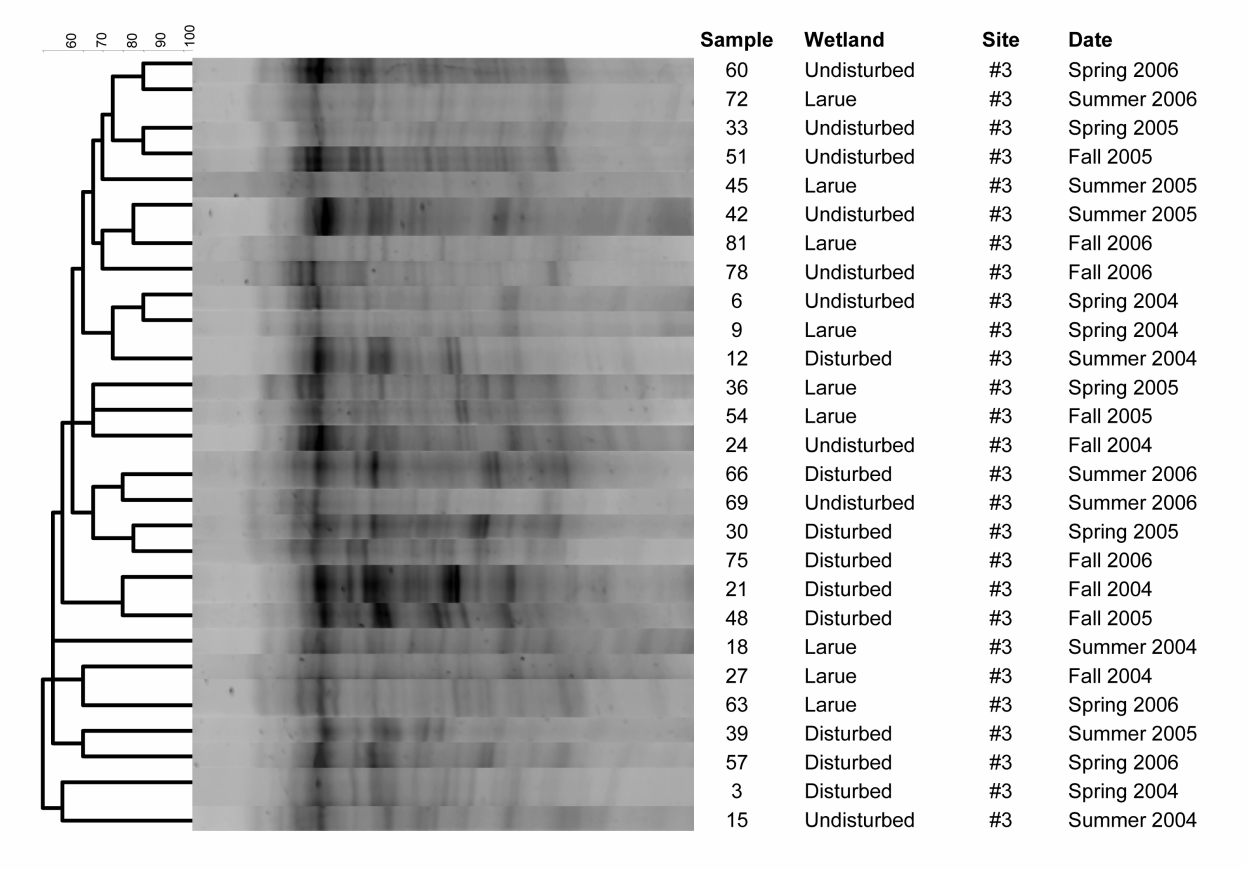

50
60
70
80
90
100
Sample Wetland Site Date
64 Disturbed #1 Summer 2006
79 Larue #1 Fall 2006
34 Larue #1 Spring 2005
70 Larue #1 Summer 2006
16 Larue #1 Summer 2004
25 Larue #1 Fall 2004
10 Disturbed #1 Summer 2004
7 Larue #1 Spring 2004
28 Disturbed #1 Spring 2005
22 Undisturbed #1 Fall 2004
46 Disturbed #1 Fall 2005
19 Disturbed #1 Fall 2004
73 Disturbed #1 Fall 2006
49 Undisturbed #1 Fall 2005
4 Undisturbed #1 Spring 2004
13 Undisturbed #1 Summer 2004
76 Undisturbed #1 Fall 2006
58 Undisturbed #1 Spring 2006
31 Undisturbed #1 Spring 2005
67 Undisturbed #1 Summer 2006
1 Disturbed #1 Spring 2004
43 Larue #1 Summer 2005
52 Larue #1 Fall 2005
37 Disturbed #1 Summer 2005
55 Disturbed #1 Spring 2006
61 Larue #1 Spring 2006
40 Undisturbed #1 Summer 2005

B.



Sample	Wetland	Site	Date
23	Undisturbed	#2	Fall 2004
41	Undisturbed	#2	Summer 2005
47	Disturbed	#2	Fall 2005
20	Disturbed	#2	Fall 2004
44	Larue	#2	Summer 2005
74	Disturbed	#2	Fall 2006
29	Disturbed	#2	Spring 2005
59	Undisturbed	#2	Spring 2006
62	Larue	#2	Spring 2006
32	Undisturbed	#2	Spring 2005
65	Disturbed	#2	Summer 2006
8	Larue	#2	Spring 2004
35	Larue	#2	Spring 2005
77	Undisturbed	#2	Fall 2006
11	Disturbed	#2	Summer 2004
80	Larue	#2	Fall 2006
17	Larue	#2	Summer 2004
26	Larue	#2	Fall 2004
5	Undisturbed	#2	Spring 2004
53	Larue	#2	Fall 2005
56	Disturbed	#2	Spring 2006
2	Disturbed	#2	Spring 2004
50	Undisturbed	#2	Fall 2005
71	Larue	#2	Summer 2006
68	Undisturbed	#2	Summer 2006
14	Undisturbed	#2	Summer 2004
38	Disturbed	#2	Summer 2005

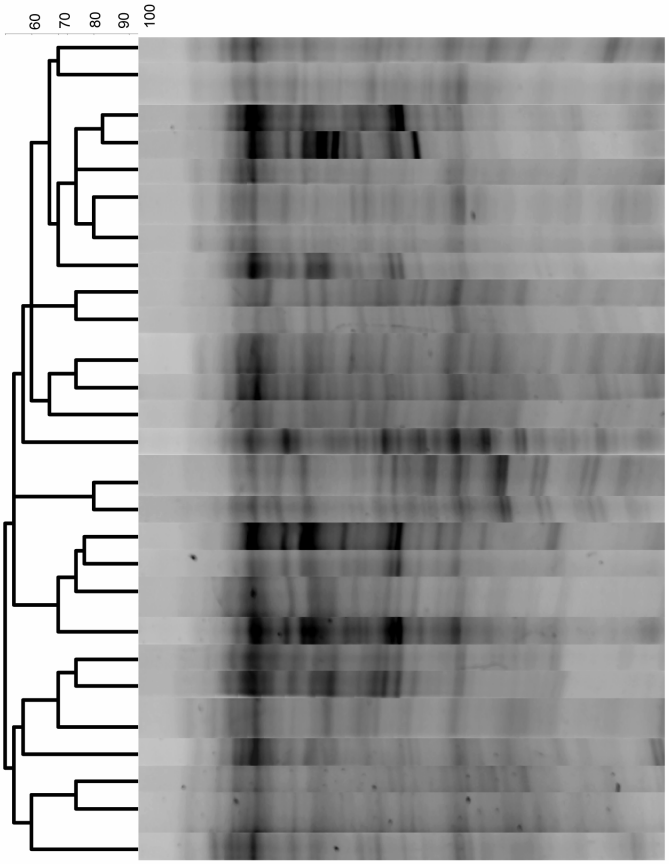
C.



Sample	Wetland	Site	Date
60	Undisturbed	#3	Spring 2006
72	Larue	#3	Summer 2006
33	Undisturbed	#3	Spring 2005
51	Undisturbed	#3	Fall 2005
45	Larue	#3	Summer 2005
42	Undisturbed	#3	Summer 2005
81	Larue	#3	Fall 2006
78	Undisturbed	#3	Fall 2006
6	Undisturbed	#3	Spring 2004
9	Larue	#3	Spring 2004
12	Disturbed	#3	Summer 2004
36	Larue	#3	Spring 2005
54	Larue	#3	Fall 2005
24	Undisturbed	#3	Fall 2004
66	Disturbed	#3	Summer 2006
69	Undisturbed	#3	Summer 2006
30	Disturbed	#3	Spring 2005
75	Disturbed	#3	Fall 2006
21	Disturbed	#3	Fall 2004
48	Disturbed	#3	Fall 2005
18	Larue	#3	Summer 2004
27	Larue	#3	Fall 2004
63	Larue	#3	Spring 2006
39	Disturbed	#3	Summer 2005
57	Disturbed	#3	Spring 2006
3	Disturbed	#3	Spring 2004
15	Undisturbed	#3	Summer 2004

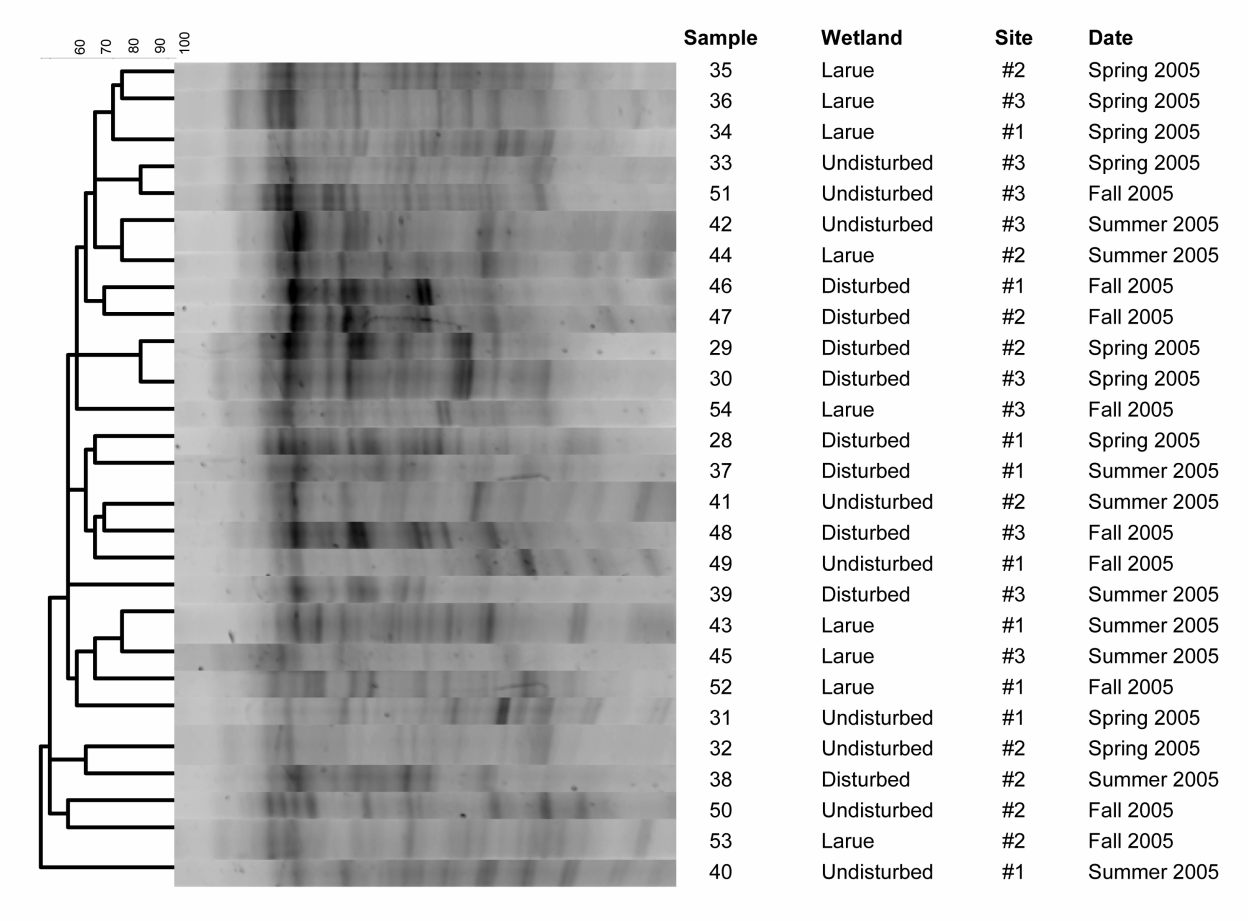
Figure 12A-C. Figure 12A (wet) appears to have higher diversity, indicating that increased water may result in increased microbial growth.

A.



Sample	Wetland	Site	Date
5	Undisturbed	#2	Spring 2004
7	Larue	#1	Spring 2004
10	Disturbed	#1	Summer 2004
11	Disturbed	#2	Summer 2004
6	Undisturbed	#3	Spring 2004
8	Larue	#2	Spring 2004
9	Larue	#3	Spring 2004
12	Disturbed	#3	Summer 2004
16	Larue	#1	Summer 2004
25	Larue	#1	Fall 2004
18	Larue	#3	Summer 2004
23	Undisturbed	#2	Fall 2004
17	Larue	#2	Summer 2004
22	Undisturbed	#1	Fall 2004
4	Undisturbed	#1	Spring 2004
13	Undisturbed	#1	Summer 2004
19	Disturbed	#1	Fall 2004
20	Disturbed	#2	Fall 2004
3	Disturbed	#3	Spring 2004
21	Disturbed	#3	Fall 2004
1	Disturbed	#1	Spring 2004
2	Disturbed	#2	Spring 2004
15	Undisturbed	#3	Summer 2004
24	Undisturbed	#3	Fall 2004
26	Larue	#2	Fall 2004
27	Larue	#3	Fall 2004
14	Undisturbed	#2	Summer 2004

B.



Sample	Wetland	Site	Date
35	Larue	#2	Spring 2005
36	Larue	#3	Spring 2005
34	Larue	#1	Spring 2005
33	Undisturbed	#3	Spring 2005
51	Undisturbed	#3	Fall 2005
42	Undisturbed	#3	Summer 2005
44	Larue	#2	Summer 2005
46	Disturbed	#1	Fall 2005
47	Disturbed	#2	Fall 2005
29	Disturbed	#2	Spring 2005
30	Disturbed	#3	Spring 2005
54	Larue	#3	Fall 2005
28	Disturbed	#1	Spring 2005
37	Disturbed	#1	Summer 2005
41	Undisturbed	#2	Summer 2005
48	Disturbed	#3	Fall 2005
49	Undisturbed	#1	Fall 2005
39	Disturbed	#3	Summer 2005
43	Larue	#1	Summer 2005
45	Larue	#3	Summer 2005
52	Larue	#1	Fall 2005
31	Undisturbed	#1	Spring 2005
32	Undisturbed	#2	Spring 2005
38	Disturbed	#2	Summer 2005
50	Undisturbed	#2	Fall 2005
53	Larue	#2	Fall 2005
40	Undisturbed	#1	Summer 2005

C.

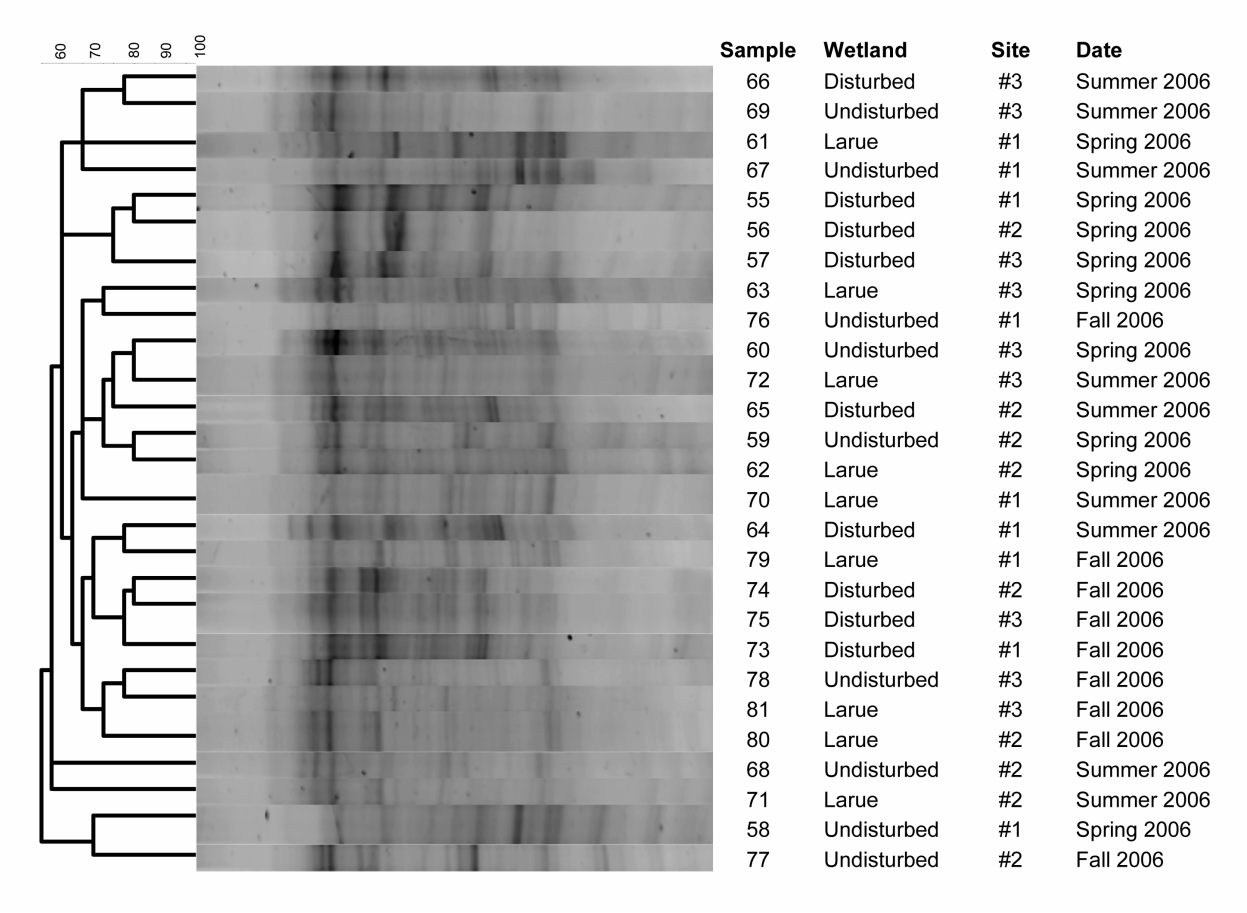


Figure 13A-C. Year dendrograms are similar, indicating that the microbial communities are stable and once a site is disturbed, that particular microbial community will be retained. Figures 12 and 13 support the conclusion that the wetland, and its inherent characteristics, has the most effect on microbial communities.